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NORTH VANCOUVER, B.C.

ENVIRONMENT CANADA 1988 CINOLA PROJECT BASELINE STUDIES

Regional Program Report No. 89-06

By

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ABSTRACT

A monitoring program was conducted over August to September 1988 to establish baseline conditions of water and sediment quality in streams adjacent to a proposed gold mine. Juvenile coho salmon that had been caged *in situ* for six weeks appeared to be an effective monitoring tool by which to establish baseline conditions of mercury availability. Feral juvenile coho salmon were also tested for muscle mercury content and the sample requirements for future trend monitoring are discussed. Liver protein and copper content of both the feral and caged juvenile coho salmon were measured in order to establish baseline conditions.

RESUME

Un programme de surveillance a été mené durant les mois d'août et septembre 1988 pour établir les conditions de base de la qualité de l'eau et des sédiments des cours d'eau adjacents à la mine d'or projettée. Des saumons coho juvéniles encagées in situ pour un durée de six semaines paraissent être un bon outil de surveillance pour établir les conditions de base de la disponibilité du mercure. Des saumons coho juvéniles sauvages furent aussi testés pour le contenu en mercure des muscles. Les besoins d'échantillonnage, pour de futurs analyses de tendance des données, sont discutés. Le contenu du cuivre et des protéines hépatiques chez les saumons coho juvéniles, aussi bien sauvage qu'encagées, ont été mesurés dans le but d'établir les conditions de base.

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SUMMARY

Several water quality characteristics such as total organic carbon, volatile residue, humic content (Aldrich equivalent) and acidity (as CaC03) were much higher at the two Barbie Creek sites and at the Florence Creek site compared to the Gold Creek site. These characteristics reflect the organic nature of the former two creeks. All three creeks were characterized by lov water hardness and alkalinity. The lovest dissolved oxygen levels were recorded in Lover Barbie Creek in early August. The highest water temperatures were recorded in Gold Creek.

Total and dissolved cadmium, copper, and lead were either near or below detectable concentrations in all streams. Barbie Creek had the highest total and dissolved arsenic concentrations. Florence Creek and Gold Creek arsenic concentrations were either near or below detectable levels. Total aluminium was higher at the two Barbie Creek sites and at the Florence Creek site compared to the Gold Creek site. Total and dissolved iron concentrations were highest at the Lover Barbie Creek site.

The routine total mercury detection limit of 0.05 ug/L was lowered to 0.005 ug/L by evaporating a 1L sample down to 0.1L. At the lover detection limit, it was evident Barbie Creek had a higher total mercury concentration compared to either Florence Creek or Gold Creek. Analytical methods that provide even lower detection limits are not routinely available but appear to be required in order to adequately characterize total mercury levels in water bodies. While not measured directly, using two seperate approaches, the methylmercury concentration of Lover Barbie Creek was estimated to be near 2.4 ng/L.

Compared to Middle Barbie Creek, Lover Barbie Creek and Gold Creek sediments were characterized by a higher organic content, lover redox potential and a higher heterotrophic bacteria count. The Lover Barbie Creek site had a higher sediment total mercury concentration than either the Middle Barbie Creek or Gold Creek sites.

Within six weeks, the caged juvenile coho salmon appeared to reflect differences in the availability of mercury between the study sites. Lower Barbie Creek and Gold Creek both had significantly higher levels of muscle tissue mercury after the six week in situ exposure. Although the surface water total mercury and sediment total mercury concentrations were higher in Lower Barbie Creek compared to Gold Creek, in this case and as reported elsewhere, they did not appear to be indicators of actual mercury availability.

The mercury accumulation rate determined in this study for Lower Barbie Creek and Gold Creek (~0.0002 ugHg/g/d) was an order of magnitude lower than that estimated from another study on the South Saskatchevan River (~0.0032-0.0048 ugHg/g/d).

Caging fish in situ is an effective monitoring tool to determine mercury availability. Fish response represents an integration of all the complex biogeochemical processes that ultimately determines the form of mercury and its availability for bioaccumulation. As such, the method would serve as a monitoring tool to assess the impact (in terms of mercury availability) of the proposed mine operation on those biogeochemical processes as well as any increased mercury loadings to the Barbie Creek drainage.

Feral juvenile coho salmon were sampled to establish baseline mercury levels for the summer period. The background tissue concentrations reflect the relative contribution of mercury from both the water and dietary components and which both vary with time of year and period of residence. The number of samples required to detect a predetermined level of increase in muscle mercury levels have been estimated for future reference.

1.0 INTRODUCTION

In June 1988, City Resources (Canada) Limited submitted a Stage II Report to the Provincial Mine Development Steering Committee (City Resources, 1988). The report outlined the proposed development of an open pit gold mine (Cinola Gold Project) on Graham Island, Queen Charlotte Islands, British Columbia. The mine would be located within the Yakoun River drainage which has significant fishery resources (Brown and Musgrave, 1979).

As part of a Cinola Gold Project pre-development data collection program, Environment Canada (Environmental Protection), undertook a monitoring program in August and September 1988. The program focused on using *in situ* caged juvenile coho salmon to establish baseline conditions from which to assess the effects on fish of potentially elevated environmental metal levels and other changes in water quality resulting from future mining activity. Water and sediment samples were collected in order to characterize the study streams. Feral juvenile coho salmon were also sampled to compare to the caged fish.

2.0 STUDY AREA

The Yakoun River drains an area of approximately 477 square kilometers. The Yakoun River flows in a northerly direction and drains into Masset Inlet near Port Clements, B.C. (Figure 1).

The tributary streams that could be potentially impacted most by the Cinola Project include Barbie Creek and Florence Creek (Figure 1). Barbie Creek drains the area surrounding the ore body (open pit) and is proposed to receive various mine related discharges (settling ponds, treated acid mine water). Upper Florence Creek has been identified as the location for the tailings impoundment. Barbie Creek drains into the Yakoun River approximately 29km. upstream of Yakoun Bay. Florence Creek drains into the Yakoun Bay estuary.

2.1 Sample Sites

Water, sediment, and fish tissue samples were collected from two sites on Barbie Creek. The Lower Barbie site (LB) was located at the downstream end of the Barbie Creek wetland and the Middle Barbie site (MB) was located upstream of the wetland (Figure 2). One site was monitored on Florence Creek (FL) for water quality only. This site was located upstream of the Main Line Road bridge (Figure 1). Gold Creek, which drains into the Yakoun River approximately 4km. upstream of Barbie Creek, was selected as a reference stream (Figure 1). Water, sediment, and fish tissue samples were collected from a site (G) located immediately downstream of Marie Lake. A description of each site is provided in Table 1.

A single grab water sample was collected in September from the Department of Fisheries and Oceans Pallant Creek hatchery water supply.

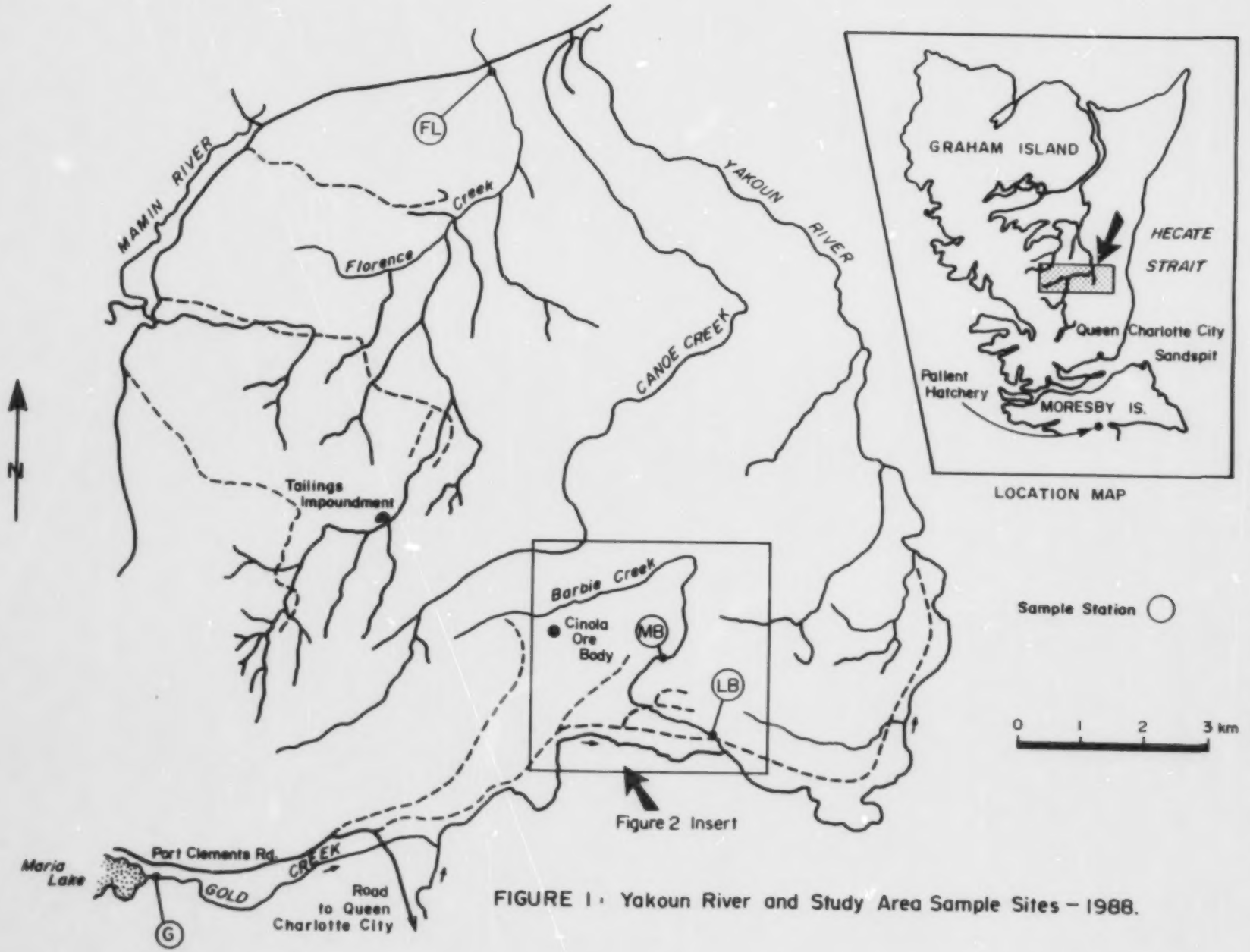

FIGURE 1 · Yakoun River and Study Area Sample Sites – 1988.

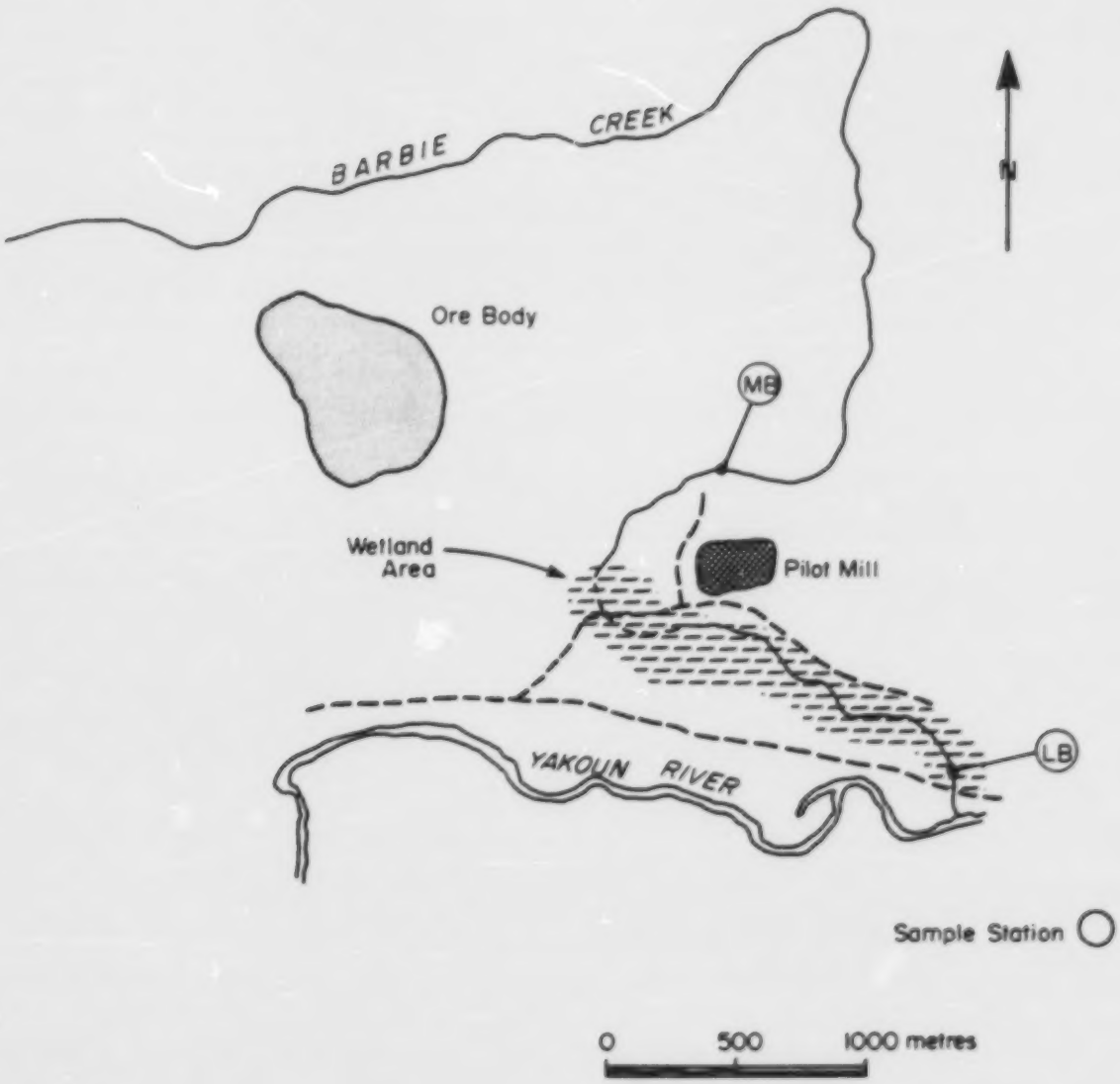

FIGURE 2. Barbie Creek Study Area and Sample Sites - 1988.

TABLE 1 : SAMPLE SITE DESCRIPTION

(see Figures 1 & 2)

SAMPLE SITE	DESCRIPTION
LOWER BARBIE CREEK (LB)	- Lower end of Barbie Creek wetland, approximately 50 meters upstream of Branch 40 road crossing. - Slow flowing channel containing pieces of tree debris (logs, bark, and branches)and with an organic substrate.
MIDDLE BARBIE CREEK (MB)	- Upstream of Barbie Creek wetland. - Generally slow flowing channel with fallen tree debris and with a sandy substrate.
FLORENCE CREEK (FL)	- Approximately 45 meters upstream of Main Line road bridge crossing. - Gravel/sandy substrate.
GOLD CREEK (G)	- Approximately 50-75 meters downstream of Marie Lake outlet. - Generally slow flowing section of creek with fallen tree debris and with an organic substrate.

3.0 MATERIALS AND METHODS

3.1 Surface Water Quality

Grab samples were collected in clean sample bottles and treated as described in Table 2. Disposable laboratory gloves were worn while the samples were being collected. Triplicate samples were collected for metal analyses. Distilled water blanks were also collected for metal analysis quality assurance.

Dissolved total phosphorus and dissolved ortho-phosphorus samples were filtered through 0.45 um distilled water soaked and rinsed cellulose acetate membrane filters. Dissolved metal samples were filtered through 0.45um cellulose nitrate membrane filters. Phosphorus samples were filtered immediately in the field. Metal samples were filtered into clean sample bottles within six hours of collection.

Samples were shipped in coolers with ice to the Environment Canada, West Vancouver Chemistry Laboratory. Humic acid samples were shipped in coolers with ice to the CB Research Laboratory in Sydney, B.C.

Analytical methods are summarized in Table 3 (Environment Canada, 1989). A more detailed description of procedure for humic acid analysis is reported in Appendix F(i).

Temperature was measured with either a hand-held thermometer or using a digital readout Hydrolab Model 4041 instrument. Dissolved oxygen samples were determined by Winkler titration or measured directly with the Hydrolab 4041 or a YSI dissolved oxygen meter.

TABLE 2 : SURFACE WATER SAMPLE CONTAINERS AND TREATMENT

ANALYSIS	SAMPLE BOTTLE & PRESERVATION
Immediates	
alkalinity acidity pH	200ml poly, cold
chloride sulfate specific conductance residue(non-filterable) (total volatile) turbidity	1000ml poly, cold
sulfide	100ml glass,0.5ml 1M zinc acetate & 0.5ml 0.5M sodium bicarbonate, cold
total organic & inorganic carbon	100ml glass, cold
humic acid	4L acid-washed amber glass, cold
dissolved oxygen	300ml glass BOD, 2ml manganese sulfate & 2ml azide solution, cold
nitrogen (total) (ammonia) (nitrite) (nitrite/nitrate)	200ml poly, cold
phosphorus (total) (dissolved)	60ml glass 60ml glass

(cont'd)

TABLE 2 cont'd: SURFACE WATER SAMPLE CONTAINERS AND TREATMENT

metals other than mercury (total and dissolved)	100ml acid washed poly, 0.5ml nitric acid
mercury (total)	100ml acid washed poly, 5ml potassium dichromate - nitric acid
(total low level)	1000ml acid washed poly, 10ml potassium dichromate - nitric acid

TABLE 3: SURFACE WATER SAMPLE ANALYTICAL METHODS

PARAMETER (detection limit)	METHOD
Immediates	
alkalinity(1mg/L)	- Potentiometric titration with sulfuric acid to pH 4.5.
acidity(1mg/L)	- Potentiometric titration with standard alkali to pH 8.3.
pH(0.1)	- Potentiometric, pH meter.
chloride(0.05mg/L)	- Colourimetric, mercuric thiocyanate-ferric nitrate combined reagent.
sulfate(1mg/L)	- Colourimetric, methylthymol blue.
specific conductance(0.1umhos/cm)	- Conductivity cell.
sulfide(0.05mg/L)	- Specific ion probe.
residues(5mg/L)	
(non-filterable)	- Gravimetric,Whatman GFC filtered and dried at 105C for one hour.
(total volatile)	- Gravimetric, evaporated at 75C overnight and then dried at 105C for one hour, loss on ignition at 550°C.
turbidity(0.1FTU)	- Nephelometric turbidity.
total organic & inorganic carbon(1mg/L)	- Combustion,infra-red
humic acid*(0.1mg/L)	- Aldrich equivalent.
dissolved oxygen(0.1mg/L)	- Winkler titration.
phosphorus(2ug/L)	
	- total and dissolved. Colourimetric, persulphate-autoclave digest, molybdate-ascorbic acid reduction.
	- dissolved ortho. Colourimetric,molybdate-ascorbic acid reduction.

cont'd

* contract analysis CB Research International Corp.

TABLE 3 cont'd : SURFACE WATER SAMPLE ANALYTICAL METHODS

nitrogen
- total(0.02mg/L). Colourimetric,persulphate
- autoclave digest, cadmium/copper reduction.
- ammonia(5ug/L). Colourimetric,phenolhypochlorite.
- nitrite(5ug/L). Colourimetric,diazotization.
- nitrite/nitrate(5ug/L). Colourimetric,cadium/copper reduction.

metals(total and dissolved). Total metal samples (except mercury) are autoclave digested with 3:1 nitric: hydrochloric acid for two hours. Mercury samples are oxidized by the addition of 2:1 sulfuric:nitric acid, 3% potassium persulfate and heated for one hour at 105°C.

- Ag(0.1ug/L) ,graphite furnace atomic absorption.
- Cd(0.1ug/L) ,graphite furnace atomic absorption.
- Cu(0.5ug/L) ,graphite furnace atomic absorption.
- Pb(0.5ug/L) ,graphite furnace atomic absorption.
- As(0.5ug/L) ,ICP emission spectrometry-hydride.
- Se(0.5ug/L) ,ICP emission spectrometry-hydride.
- Al(0.05mg/L) ,ICP emission spectrometry.
- Ca(0.1mg/L) ,ICP emission spectrometry.
- Fe(5ug/L) ,ICP emission spectrometry.
- Mg(0.1mg/L) ,ICP emission spectrometry.
- Mn(1ug/L) ,ICP emission spectrometry.
- Si(0.05mg/L) ,ICP emission spectrometry.
- Zn(2ug/L) ,ICP emission spectrometry.
- Hg(0.05ug/L**) ,cold vapour atomic absorption.

hardness(mg/L) - Calculated from dissolved metal sample.

** detection limit of 0.005ug/L when 1000ml sample evaporated (hot plate boiled) to 1/10th volume.

3.2 Sediment

Sediment samples were collected with a 3.5mm. ID acrylic core tube. A wooden dowl with a rubber bung fixed to the end of it was used to extrude the sample.

Five composite sediment samples were collected at each site for metal, volatile residue, total phosphorus and total nitrogen analyses(<0.15mm fraction). Each composite was made up of two cores. The top 5cm. of each sediment core was extruded into a clean stainless steel bowl. The sediment in the bowl was thoroughly mixed and then spooned (plastic spoon) into a kraft paper sediment bag. A subsample from each of the composite samples was spooned into a seperate kraft sample bag for sulfur and total Kjehdal nitrogen analyses (whole sample). Sediment samples were frozen in the field with dry ice.

Sediment sample analyses and analytical methods are summarized in Table 4. The samples were analyzed at the Environment Canada West Vancouver Laboratory or in the case of sulfur and Kjehdal nitrogen, under contract at the Chemex Laboratory in North Vancouver. National Research Council sediment reference sample MESS1 was used to determine metal recovery .

An additional sediment sample was collected for heterotophic bacteria analysis (Appendix G). Between stations, the core tube and bowl were rinsed with 70% ethanol and distilled water and either air-dried or wiped dry with a paper towel. A sterile plastic spoon was used to transfer the sediment into a sterilized polyethylene sample bottle. The samples were kept cold and shipped to the Environment Canada Microbiology Laboratory in North Vancouver.

Two to three sediment samples were collected for redox potential analysis. After collection, the core sample was allowed to settle for approximately two minutes. The sample was then slowly extruded to within 1cm. of the top of the core tube. The top 1cm. of water and surficial sediment was then poured into a clean 50ml. polyethylene centrifuge tube and capped. Redox measurements were made within five minutes of the samples being collected. Redox meaurements were made with a Metrohm model E588 pH/Redox meter.

TABLE 4 : SEDIMENT ANALYSES AND ANALYTICAL METHODS

Volatile Residue - Sample is oven dried at 90 C overnight, oven dried at 103 C for one hour and then muffled at 550 C for one hour.
- Gravimetric analysis.

Metals - Samples are oven dried at 40 C, sieved to <0.15mm. and then rolled to homogenize. The sample is then weighted (0.3g) into a Teflon digestion vessel and digested with 4.5ml HNO3 and 1.5ml HCl and 1ml deionized water in a microwave oven (720 joules/sec) for 15 minutes. The sample is cooled, volumized, and settled overnight. The decant is analyzed.

- Ag(2ug/g)*,	ICP emission spectrometry
- Al(8ug/g),	ICP emission spectrometry
- As(8ug/g),	ICP emission spectrometry
- Ba(0.2ug/g),	ICP emission spectrometry
- Ca(20ug/g),	ICP emission spectrometry
- Cd(0.8ug/g),	ICP emission spectrometry
- Cr(0.8ug/g),	ICP emission spectrometry
- Cu(0.8ug/g),	ICP emission spectrometry
- Fe(8ug/g),	ICP emission spectrometry
- Hg(0.008ug/g),	cold vapour atomic absorption
- Mg(20ug/g),	ICP emission spectrometry
- Mn(0.2ug/g),	ICP emission spectrometry
- Ni(3ug/g),	ICP emission spectrometry
- Pb(8ug/g),	ICP emission spectrometry
- Si(8ug/g),	ICP emission spectrometry
- V(2ug/g),	ICP emission spectrometry
- Zn(0.3ug/g),	ICP emission spectrometry

* detection limit for 0.3g dried sample

Heterotrophic Bacteria - Surface or plate count on heterotrophic plate count agar.

Total Nitrogen (.05mg/g) - 0.015-0.03g sample, persulphate-autoclave digestion for one hour.
- Colourimetric, cadmium/copper reduction.

cont'd

TABLE 4 cont'd : SEDIMENT ANALYSES AND ANALYTICAL METHODS

Total Kjehdal Nitrogen*(0.01ug/g)	- prep code 268 - Nesslerization.
Total Sulfur*(0.01mg/g)	- prep code 268 - Leco induction furnace.
Sulfate-sulfur(0.1mg/g)	- prep code 268 - hydrochloric acid soluble sulfate, gravimetric.
Sulfide-sulfur	- by calculation, Total sulfur minus acid soluble sulfate sulfur.

* contract analyses by Chemex Labs Ltd.

3.3 Fish Studies

3.3.1 Caged Fish Emplacement and Sampling. Juvenile coho salmon were obtained from the Department of Fisheries and Oceans Pallant Creek hatchery located on Moresby Island (Figure 1). The fish were initially transported from the hatchery to Queen Charlotte City on Graham Island in a 300L polyethylene tank at ambient water temperature. The tank was oxygenated during transport. The fish were subsequently transfered to smaller 60L coolers lined with polyethylene liners. Ice packs were placed under the liners and the coolers were oxygenated during transport to the study sites. In total, the fish were in transit for approximately 6 hours. Temperature and dissolved oxygen levels were recorded during transport to the study sites.

The fish cages were constructed of Aqua Mesh (13mm. x 13mm. opening, plastic coated metal screen) and were lined with Vexar (7mm. opening) to prevent any escapement. Cage dimensions were 30.5cm. x 30.5cm. x 122cm. (110L volume). The cages were steam-cleaned prior to use.

The fish cage sites were all characterized by slow current velocities (<11 cm/s). Current velocity was measured with a Marsh McBirney velocity meter. With the exception of one cage at the Gold Creek site which had 28 fish, 30 fish were placed in each of two cages per site. The loading per cage was approximately 3.3g/L. The cages were suspended just below the water surface and were not in contact with the stream bottom. The fish were fed (4% of the total fish wet weight per cage) three times a week with a commercial fish ration (Biodiet). The ration was sprinkled slowly over the upstream end of the cage. The cages were cleaned with a plastic bristle brush after each feeding to remove excess ration and any other accumulated matter. The fish were held *in situ* for six weeks over August 5 to September 16 1988.

Whole fish samples were collected for tissue analyses at the time the fish were initially picked up at Queen Charlotte City (Day-0) and from the study sites after six weeks of caging (Day-42). The hatchery fish were resampled after six weeks. In each case, individual fish were quickly netted,

placed in individual labelled whirl pac bags and then immediately placed in a chest cooler containing dry ice. Five composite samples of 10 fish each were collected from each cage site for liver tissue analyses. The remaining fish were similarily handled and to be used for muscle tissue mercury analyses. When in transit the fish were maintained frozen with dry ice. The samples were stored at -20° C.

3.3.2 **Feral Fish Collection**. Norecol Consultants used baited G-traps to collect feral juvenile coho salmon from Barbie Creek and Gold Creek over August 16-17 1988 and September 14-17 1988. During each trap inspection, the larger juvenile coho were collected and placed in individual labelled whirl pac bags. The fish were immediately placed in a chest cooler containing dry ice. The fish were subsequently provided to Environment Canada and handled in an identical manner to the caged fish.

3.3.3 **Hatchery Reference Fish and Caged Fish Tissue Analyses**. The caged fish were seperated into two groups. The first group consisted of eight fish each from the initial reference sample (Pallant Hatchery Day-0), Lower Barbie Creek (Day-42), Middle Barbie Creek (Day-42) and Gold Creek (Day-42). This group was used for muscle tissue mercury analyses only. Individual fish were partially unthawed, wiped dry with a paper towel, and fork length (0.1cm) and weight(0.1g) were recorded.

The larger second group of fish was submitted to CB Research International for liver tissue analyses. After CB Research had completed their analyses, samples of hatchery reference fish (Day-0 and Day-42) were obtained for additional muscle tissue mercury analyses.

3.3.3.1 **Muscle tissue - mercury**. The fish were partially unthawed and then carefully wiped clean with Kimwipe tissue paper. During each dissection and between individual samples, the dissecting tools (scalpel and forceps) were repeatedly rinsed in a series of 5% nitric acid and deionized water solutions and dried with Kimwipe tissue paper. Rinse solutions and scalpel blades were renewed between the groups of fish from each site.

Above the lateral line muscle tissue samples (no skin or bone) for individual fish were dissected, placed in ziploc polyethylene sample bags, and refrozen until analyzed for mercury at the Environment Canada West Vancouver Laboratory .

Sample moisture content was determined by weighting the sample before and after freeze-drying. Freeze-dried samples were ground and then weighted (nominal 0.3g sample) into Teflon vessels. Nitric acid (5ml) was added and the samples were left to sit for one hour. The samples were then microwave digested (720 joules/sec) for 15 minutes, cooled, and volumized with 1ml of hydrochloric acid and 20ml of deionized water. The samples were transferred to acid-washed 30ml polyethylene sample bottles and allowed to degas for one week prior to analysis. Mercury was analyzed by cold vapour atomic absorption spectrometry. Reference samples TUNA-50 (muscle tissue) and TORT-1 (lobster hepatopancreas) were used to determine mercury recovery for the procedures used.

3.3.3.2 Liver tissue - protein fractions and copper. Liver tissue samples were dissected and analyzed under contract by CB Research International Corp. Their report including analytical methods is presented in Appendix F(i).

Liver tissue samples were analyzed for total protein (whole homogenate and total cytosol fractions) and polarographically active protein (cytosol-thiolic and denatured ethanol extract - metallothionein fractions). Various liver tissue fractions (whole homogenate, pellet material from centrifugation, cytosol and the denatured ethanol extract) were also analyzed for copper. The hatchery control (Day-0), Lover Barbie Creek (Day-42), Gold Creek (Day-42) fish were analyzed for copper.

3.3.4 Feral Fish Tissue Analyses. Feral fish were seperated into composite samples of 10 fish each for liver tissue protein analyses. Enough fish were caught for five composite samples from Lover Barbie Creek in September. Two composite samples were obtained from Lover and Middle Barbie

Creek in August and from Gold Creek in September. One composite sample was collected from Gold Creek in August. These samples were treated in a manner identical to that of the caged fish. The Lower Barbie Creek (September) fish were analyzed for copper.

After CB Research had completed their analyses, fish samples were obtained and dissected for muscle tissue mercury analyses. Eight samples from Lower Barbie Creek in August and September, Middle Barbie Creek in August, and Gold Creek in September were analysed. These fish were treated in a manner identical to that of the caged fish except individual samples consisted of a composite of two fish.

3.4 Fish Transportation Study

To investigate whether the transportation of fish, as conducted in this study, has any influence on liver protein levels, a short term study was conducted in September when the hatchery fish were being resampled. Fish were transported under conditions similar to those in August. The coolers were maintained at ambient temperature and were oxygenated. The effect of elevated temperature was also assessed. A second set of coolers were oxygenated but water temperature was elevated over the period of transport by placing containers of hot water under the cooler liners.

The fish samples were collected and treated in the manner already described.

4.0 RESULTS

4.1 Surface Water Quality and Stream Velocity

The water quality results for the four stream stations (MB, LB, FL, and G) are reported in Appendix A(i) (non metals) and Appendix A(ii) (metals). The total cadmium, total copper, total lead and total zinc results for the August 23 1988 survey have not been reported. The deionized water reference blank sample indicated an inexplicable contamination problem with these metals.

Stream velocities are reported in Appendix A(iii).

The Pallant Creek hatchery water intake quality is reported in Appendix A(iv).

4.2 Sediment Quality

The sediment quality results for the three stream stations (MB, LB,and G) are reported in Appendix B(i) (non metals) and Appendix B(ii) (metals). The reference sediment results are reported in Appendix B(iii).

4.3 Juvenile Coho Salmon Muscle Tissue Mercury

4.3.1 Caged Fish. The muscle tissue mercury and moisture content results and fish weight, fork length and condition factor for the caged fish are reported in Appendix C(i). The coefficient of condition was calculated as 100 times wet wt. (g) divided by fork length (cm.) cubed (Reimer, 1963). The somatic index was calculated as liver wet wt. divided by fish wet wt. times 100. Growth rate was calculated as: log (base e) mean wt. Day-42 minus log (base e) mean wt. Day-0 times 100 (Davis, 1978). The reference tissue results are reported in Appendix C(iii). The muscle tissue mercury levels for the Pallant hatchery fish at Day-0 and at Day-42 are reported in Appendix C(iv).

4.3.2 **Feral Fish.** The muscle tissue mercury and moisture content results and fish weight, fork length, and condition factor for the feral fish are reported in Appendix C(ii). The reference tissue results are reported in Appendix C(iii).

4.4 Food Ration Quality

The quality of the fish ration used at the Pallant Creek hatchery and that used to feed the caged fish is reported in Appendix D.

4.5 Fish Transportation

The dissolved oxygen content and temperature of the water in the transportation containers during transit to the study sites (August 5 1988) and during the short term heat study (September 15 1988) are reported in Appendix E.

4.6 Liver Tissue Protein Fractions and Copper

The liver protein analyses for the various fish groups are summarized in Appendix F(ii) (caged fish) and F(iii) (feral fish). The copper concentrations for the four test groups are reported in Appendix F(i) Table (5.3B).

5.0 DISCUSSION

5.1 Surface Water

The water quality characteristics of the study sites are summarized in Table 5. The humic nature of Barbie and Florence Creeks relative to Gold Creek is reflected by their higher organic carbon, volatile residue, and humic (Aldrich equivalent) content as well as higher acidity. The nature of organic carbon in natural waters has been described in detail by Thurman, 1985.

All the streams were characterized by low water hardness and alkalinity. The pH of Barbie Creek was lower than that of Florence Creek and Gold Creek, Gold Creek being the highest. Dissolved oxygen levels were lowest in Lower Barbie Creek and saturation levels ranged between 59%-94% (mean = 71%). Gold Creek had the highest mean water temperature (17.3°C). For this study, detectable total mercury concentrations were obtainable if a 1L sample was evaporated to 0.1 volume and a 0.1 detection limit (5ng/L) was applied. Barbie Creek had higher total mercury levels than either Florence Creek or Gold Creek. The single-stage gold amalgamation preconcentration procedure used by Agassiz North Associates, 1988 to measure total mercury in Barbie Creek and Florence Creek provides an even lower detection limit (0.7ng/L).

Arsenic was detectable in Barbie Creek and at or near the detection limit in Florence and Gold Creeks. Cadmium, copper, and lead were near or below detectable levels at all sites. Zinc was below the detection limit in Florence and Gold Creeks. Zinc was detectable in Barbie Creek but appeared to be highly variable. Iron and aluminium were higher in Barbie and Florence Creeks compared to Gold Creek.

TABLE 5 : SUMMARY OF MEAN WATER QUALITY CHARACTERISTICS OF BARBIE, FLORENCE, AND GOLD CREEKS

	BARBIE		FLORENCE	GOLD
Non Metals	LB	MB	FL	G
pH	6.6	6.5	7.0	7.3
acidity(mg/L)*	5.7	6.0	5.0	1.9
alkalinity(mg/L)*	7.8	7.1	10.6	13.3
hardness(mg/L)*	12.7	13.3	10.8	14.1
chloride(mg/	7.7	7.9	6.2	3.6
conductivity(umho/cm)	51	53	43	44
sulphate(mg/L)	11	11	7	3
T phosphorus(ug/L)	55	44	37	12
TD phosphorus(ug/L)	24	25	22	4
ammonia(ug/L)	33	19	15	<9
nitrite(ug/L)	<5	<5	<5	<5
nitrite-nitrate(ug/L)	<7	<6	<5	<5
T nitrogen(mg/L)	0.58	0.42	0.36	0.15
T organic carbon(mg/L)	28	25	25	5
T inorganic carbon(mg/	4	2	3	4
humic content(mg/L)**	52	47	41	5
non-filterable residue(mg/L)	12	11	<1	<5
volatile residue(mg/	63	60	48	<10
temperature(°C)	14.8	12.9	13.2	17.3
dissolved oxygen(mg/L)	7.0	8.6	9.6	9.9
oxygen saturation(%)	71	84	94	106

cont'd

* as CaCO3
** as Aldrich equivalent

TABLE 5 cont'd: SUMMARY OF MEAN WATER QUALITY CHARACTERISTICS OF BARBIE, FLORENCE, AND GOLD CREEKS

	BARBIE		FLORENCE	GOLD
Metals	LB	MB	FL	G
THg(ng/L)	<16	12	<7	<5
TAg(ug/L)	<0.1	<0.1	<0.1	<0.1
DAg(ug/L)	<0.1	<0.1	<0.1	<0.1
TAs(ug/L)	7.8	4.0	<0.5	<0.5
DAs(ug/L)	3.0	3.3	<0.5	<0.8
TSe(ug/L	<0.5	<0.5	<0.5	<0.5
DSe(ug/L)	<0.5	<0.5	<0.5	<0.5
TCu(ug/L	<0.5	<0.7	<0.5	<0.5
DCu(ug/L)	<0.5	<0.5	<0.5	<0.5
TCd(ug/L)	<0.1	<0.1	<0.1	<0.1
DCd(ug/L)	<0.1	<0.1	<0.1	<0.1
TPb(ug/L)	<0.5	<0.5	<0.5	<0.5
DPb(ug/L)	<0.5	<0.5	<0.5	<0.5
TZn(ug/L	<5	<5	<2	<2
DZn(ug/L)	<4	5	<2	<2
TCa(mg/L)	3.2	3.3	2.4	4.5
DCa(mg/L)	2.9	3.1	2.2	4.4
TMg(mg/L)	1.3	1.3	1.3	0.8
DMg(mg/L)	1.2	1.2	1.2	0.7
TFe(mg/L)	5.056	2.543	1.843	0.201
DFe(mg/L)	1.496	1.131	0.968	0.111
TMn(mg/L)	0.151	0.155	0.032	0.022
DMn(mg/L)	0.113	0.140	0.020	0.009
TAl(mg/L)	0.66	0.74	0.55	0.09
DAl(mg/L)	0.37	0.56	0.43	<0.07

T = total, D = dissolved

5.2 Sediment

The sediment quality characteristics of the study sites are summarized in Table 6. Compared to Middle Barbie Creek, Lower Barbie Creek and Gold Creek sediments had a higher organic content (SVR), lower redox potential, higher sulphide content and higher heterotrophic bacteria count. Middle Barbie Creek sediments were composed largely of coarse sand and the organic content of the <0.15mm fraction probably reflects a higher organic content than would the whole sample. Sediment mercury levels were highest at the Lower Barbie Creek site.

Bjornberg et al., 1988 theorized that the activity of divalent mercury (Hg+2) in natural waters is essentially regulated by the activity of sulphide ions, which, in turn, is strongly affected by pH and redox conditions. During bacterial decomposition, oxygen is consumed and carbon dioxide (and bacterial biomass) is created. A link exists between the oxic conditions, the redox conditions, and the activity of the sulphide ion (Bjornberg et al., 1988). Jackson, 1988 identified three sites that represented a regular progression from normal preimpoundment (reservoir formation) conditions to an environment affected to the greatest degree by recently drowned soil and vegetation. He demonstrated the link between abnormally high rates of monomethylmercury (CH_3Hg^+) production in sediments (high mercury methylation capability) with intensified heterotrophic microbial activity (measured as carbon dioxide and methane production) and elevated organic concentrations accompanied by oxygen depletion and reducing conditions. Mercury methylation capability was not significantly correlated with total sediment mercury.

Ramlal et al., 1986 described methods for measuring specific rates of mercury methylation and degradation (M/D ratio). They reported that the observed M/D ratio was consistently higher at sites where organic rich flooded soils were sampled. The specific rate measurements do provide the means to determine whether certain environmental perturbations will tend to increase or

TABLE 6 : SUMMARY OF SEDIMENT QUALITY CHARACTERISTICS OF BARBIE AND GOLD CREEKS

	BARBIE		GOLD
Non Metals*	LB	MD	G
(whole sample)			
redox(mV)	+60	+350	+130
heterotrophic bacteria(CFU/g)	99000	63000	242000
sulphide-S(mg/g)	2.20	<0.07	0.69
TKN (mg/g)	5.62	0.45	2.46
(<0.15mm fraction)			
TN(mg/g)	6.5	1.9	6.2
TP(mg/g)	1.04	0.88	0.92
SVR(%)	28.5	13.8	24.0
As(ug/g)	68	68	56
Hg(ug/g)	0.26	0.11	0.15
Cd(ug/g)	1.4	1.2	1.2
Cu(ug/g)	17.1	17.1	16.4
Zn(ug/g)	217	122	116
Al(mg/g)	25.0	24.7	25.8
Fe(mg/g)	37.6	46.4	37.5

* mean values except for redox potential which is the median value

decrease methylating or demethylating activity (Ramlal et al., 1986). Parks et al., 1986 reported that mercury concentrations in water can fluctuate seasonally by an order of magnitude and highest concentrations are associated with elevated summer temperatures.

Ford and Naiman, 1988 reported that beaver apparently influence the biogeochemical cycling of carbon by creating conditions for sediment accumulation in streams (increased organic content), providing anoxic conditions (low oxygen and low redox potential) suitable for significant methanogenesis (methane production). Barbie Creek and other creeks on the Queen Charlotte Islands can be influenced by beaver activities. The above study demonstrates that many factors can influence biogeochemical cycles and ultimately the environmental conditions that could promote or inhibit the availibility of mercury for bioaccumulation by aquatic organisms.

In a study conducted independently of the work described herein, Agassiz North Associates, 1988 reported that Lower Barbie Creek and Gold Creek (caged fish sites) supported significant levels of net microbial mercury methylation (high M/D). Middle Barbie Creek (caged fish site) had a low methylation balance (low M/D).

5.3 Fish Studies

5.3.1 Caged Fish Condition. No mortalities occurred over the six week caging period. The fish appeared to be in good condition physically and there was no evidence of fin or body abrasion. Observations of stomach contents indicated that the fish were feeding on the ration provided as well as some aquatic insects.

Several measures of "general" fish condition were monitored as indicators of how the fish responded to caging. These indicators can be compared to the hatchery stock over the same period (Table 7).

TABLE 7 : INDICATORS OF CAGED FISH CONDITION
(mean and sd)

	Pallant Hatchery		Barbie Creek		Gold Creek
	Day-0	Day-42	LB Day-42	MB Day-42	G Day-42
(fork length-cm)					
	8.9	10.6*	9.5*	9.6*	9.6*
	(0.7)	(0.7)	(0.5)	(0.6)	(0.7)
(weight-g)					
	11.7	17.1*	11.6	12.4	12.6
	(2.7)	(3.3)	(2.0)	(2.0)	(2.1)
(liver wet wt-g)					
	0.17	0.20	0.13*	0.12*	0.13*
	(0.07)	(0.08)	(0.05)	(0.04)	(0.03)
(condition factor)					
	1.6	1.4*	1.3*	1.4*	1.4*
	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)
(somatic index)					
	1.4	1.1*	1.1*	1.0*	1.0*
	(0.4)	(0.4)	(0.3)	(0.3)	(0.2)
(specific growth rate - %)					
		+0.90	-0.02	+0.14	+0.17

* Tukey's Multiple Comparison Test. Significantly different compared to reference Day-0 (p=0.05, n=50). Condition Factor and Somatic Index analysis was on arcsine transformed data. Zar, 1984.

The condition factor (measure of relative fatness) of the caged fish was lower at Day-42 compared to Day-0. The same was observed for the hatchery stock. For the caged fish, the lower condition factor appears to reflect an increase in length, while weight remained the same or increased slightly. For the hatchery stock, the fish gained weight as well as length resulting in a net reduction in the condition factor. Reimer, 1963 reported that most hatchery-reared rainbow trout lose weight steadily after beginning stream life and that the lose of weight was reflected by a lower condition factor. The specific growth rate indicated that while the caged fish were not groving as they did under hatchery conditions, some growth was observed at two of the sites, indicating exposure conditions were satisfactory.

5.3.2 **Caged Fish Mercury**. The mean muscle mercury levels for the fish from the caged sites and the control fish (hatchery Day-0 and Day-42) are reported in Table 8. The mean muscle mercury level for the Lower Barbie Creek and Gold Creek fish are significantly different than at Day-0. The Middle Barbie Creek fish mercury level was not significantly different. The results indicate that caging hatchery reared fish *in situ* is an environmental effects monitoring tool that can effectively distinquish small differences in the availability of mercury in water and ultimately, bioaccumulation potential. The method used to determine total mercury in water in this study reflected more mercury in Barbie Creek compared to Gold Creek and the sediment mercury levels were highest in Lower Barbie Creek. In this case, neither of these appeared to be an indicator of actual mercury availability. Elwood et al., 1987, reported that their results indicated that total mercury in sediments and water are not reliable variables for predicting the rate of mercury uptake by fish. As discussed previously, a combination of biogeochemical factors ultimately determine the availability of mercury. The actual accumulation potential would largely be determined by a combination of exposure period, diet, and time of year.

TABLE 8 : MUSCLE TISSUE MEAN MERCURY LEVELS IN CAGED AND HATCHERY JUVENILE COHO SALMON

CAGED AND HATCHERY FISH (ugHg/g wet wt)

	Reference Pallant	Hatchery	Lower Barbie	Middle Barbie	Gold
	Day-0	Day-42	Day-42	Day-42	Day-42
Mean	0.057	0.056	0.066*	0.060	0.066*
SD	0.004	0.006	0.005	0.004	0.005
RSD(%)	7	11	8	7	8
n	8	8	8	8	8

* Tukey's Multiple Comparison Test. Significantly different compared to reference Day-0 (p=0.05). Zar 1984.

Phillips and Buhler, 1978 reported that mercury concurrently accumulated from food and water was quantitatively additive. Accumulation rates from food or water, when summed, nearly equaled that from both sources presented together. As well, mercury was taker up at a constant rate by fish (rainbow trout,3-10g) exposed to methylmercury in their diet, in the water, or in both media simultaneously. Food consumption rate (greater growth with greater food consumption) did not influence the rate of mercury accumulation by fish experiencing the same methylmercury concentration in water (Phillips and Buhler, 1978). Phillips and Buhler, 1978 reported that the relationship between the concentration of methylmercury in water (Xw = ugHg/L), methylmercury consumption rate (Xc = ngHg/g per day), and mercury accumulation rate (Y= ugHg/g per day) is described by the regression equation $Y = 0.084Xw + 0.00068Xc$.

The mercury accumulation rate (Y) for the Lower Barbie Creek and Gold Creek caged fish was determined to be ~0.0002 ugHg/g per day. This was calculated from the increase in the mean muscle Hg content (ugHg/g) divided by the exposure period (42 days). The Phillips and Buhler regression can then be applied to estimate the methylmercury concentration in water by assuming the methylmercury consumption rate is zero (i.e. the fish ration provided represents an insignificant level of contamination). The methyl mercury concentration in water ($Xw = Y/0.084$) is estimated to be 2.4ng/L.

Caged fish have been used successfully as a monitoring tool to assess the availability of mercury (Hasselrot, 1968; Uthe et al., 1973; Rudd and Turner, 1983; Elwood et al., 1987). However, accumulation rates have not been reported in these studies. In order to put the accumulation rate reported in this study into perspective, accumulation rates were estimated from the data presented by Uthe et al., 1973. Uthe et al., 1973 caged rainbow trout in several locations of the South Saskatchewan River. At the control sites (Diefenbaker Lake and Pike Lake, respectively), in Summer 1970, the uptake rates were 0.0048 and 0.0033 ugHg/g/d respectively after six weeks exposure and 0.0032 and 0.0032 ugHg/g/d respectively after eight weeks exposure. These rates are an order of magnitude higher than those measured in this study.

Uthe et al., 1973 clearly demonstrated seasonal differences in the accumulation of mercury (highest in summer) as well as differences in the same season for different years.

The proportion of methylmercury to total mercury has been reported to be approximately 30% for several river systems (Kudo et al., 1982; Schintu et al., 1989). Assuming this general observation can be applied elsewhere, then by using the mean Barbie Creek background total mercury concentration of 7.9 ng/L (Agassiz North Associates, 1988), the estimated methyl mercury concentration for Barbie Creek would be 2.4 ng/L. This concentration is identical to that obtained with the regression analysis. An analysis of the background methylmercury concentration of Barbie and Gold Creeks would have been of interest in this case but the procedure is not one that is routinely available.

5.3.3 Feral Fish Mercury. The mean muscle mercury levels for the fish from Barbie Creek and Gold Creek are reported in Table 9. No significant differences were found between fish collected from Lower Barbie Creek in August compared to September. No significant differences were found between fish collected from Lower Barbie Creek and Middle Barbie Creek in August. Without knowing the relative contribution of mercury from both the water and dietary components, as well as differences in seasonal effects and period of exposure, it is not possible to project what a typical "background" concentration might be.

TABLE 9 : MUSCLE TISSUE MEAN MERCURY LEVELS IN FERAL JUVENILE COHO SALMON

	Feral Fish (ugHg/g wet wt)			
	Lower Barbie		Middle Barbie	Gold
	August	September	August	September
Mean	0.193	0.171	0.184	0.134
SD	0.036	0.034	0.025	0.060
RSD(%)	19	20	14	45
n	8	8	8	8

The larger relative standard deviation (RSD) observed for the Gold Creek fish may be in part due to sampling fish with different exposure backgrounds. Namely, truly feral fish spawned and reared in Gold Creek and fish introduced at some time from the Marie Lake CEDP hatchery .

For future trend monitoring it would be advantagous to predetermine the level of change one desires to be able to detect. The number of samples (n) required to detect that change will largely be determined by the variability associated with the sample mean (estimate of population mean), hov small a change one wishes to measure and the chance with which one desires to be able to detect that change.

As an example, all of the feral fish collected from Barbie Creek were used to determine the sample size required to measure a one-half standard deviation increase and a one standard deviation increase in the mean muscle tissue mercury level (Table 10).

TABLE 10 : ESTIMATED SAMPLE SIZE TO MEASURE A SPECIFIED LEVEL OF CHANGE IN BARBIE CREEK FERAL FISH MERCURY CONCENTRATIONS

Background Information	Percent Chance	Specified Change	Estimated n*
mean = 0.183 ugHg/g sd = 0.034 var = 0.0012 n = 24	(i) 90%	0.034 ugHg/g	13
	(ii) 90%	0.017 ugHg/g	46

* Estimation of required n to test Ho: U = Uo, p=0.05 (Zar,1984 pg. 110)

With a 90% chance of detecting a mean significantly different by as little as 0.034 ugHg/g, the estimated sample size is n=13. To measure a 0.017 ugHg/g increase the estimated sample size is n=46.

5.3.4 Liver Protein and Copper. The reader is referred to Appendix F(i) for the detailed discussion of these data. The liver protein analyses and the distribution of copper associated with the various protein fractions should aid in the assessment of the effects on fish from any future effluent discharge(s) to Barbie Creek.

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APPENDIX A - WATER QUALITY (i) NON METALS

- pH, acidity, and alkalinity
- chloride, specific conductance, sulphate, and sulphide
- phosphorus
- nitrogen
- carbon and humic content
- residues and turbidity
- temperature and dissolved oxygen
- hardness

APPENDIX A(1) - pH, ACIDITY AND ALKALINITY

DATE (1988)	STATION			
pH	LOWER BARBIE	MID BARBIE	LOWER FLORENCE	GOLD
AUG 3	6.5	6.5	7.2	
AUG 6	6.6	6.7	7.0	7.4
AUG 23	6.5	6.4	6.9	7.3
SEP 14	6.8	6.5	6.9	7.3
MEAN	6.6	6.5	7.0	7.3
SD	0.1	0.1	0.1	0.0
n	4	4	4	3
ACIDITY(mg/L as CaCO3)				
AUG 3	4.7	4.7	3.5	
AUG 6	4.7	4.7	3.9	1.6
AUG 23	9.4	9.4	6.3	2.4
SEP 14	4.2	5.1	6.3	1.7
MEAN	5.7	6.0	5.0	1.9
SD	2.1	2.0	1.3	0.4
n	4	4	4	3
ALKALINITY(mg/L as CaCO3)				
AUG 3	7.5	7.0	13.0	
AUG 6	8.0	8.0	10.0	13.0
AUG 23	8.0	6.5	10.0	14.5
SEP 14	7.5	7.0	9.5	12.5
MEAN	7.8	7.1	10.6	13.3
SD	0.2	0.5	1.4	0.8
n	4	4	4	3

APPENDIX A(i) - CHLORIDE, SPECIFIC CONDUCTANCE, SULPHATE, AND SULPHIDE

DATE (1988)	STATION			
CHLORIDE (mg/L)	LOWER BARBIE	MID BARBIE	LOWER FLORENCE	GOLD
AUG 3	8.4	8.5	6.9	
AUG 6	9.0	9.1	7.3	4.9
AUG 23	5.6	6.0	4.3	2.3
SEP 14	7.8	7.9	6.4	3.6
MEAN	7.7	7.9	6.2	3.6
SD	1.3	1.2	1.2	1.1
n	4	4	4	3
CONDUCTIVITY (umho/cm)				
AUG 3	49	53	41	
AUG 6	53	55	44	44
AUG 23	48	53	41	43
SEP 14	53	53	44	44
MEAN	51	53	43	44
SD	2	1	2	0
n	4	4	4	3
SULPHATE (mg/L)				
AUG 3	12	10	7	
AUG 6	12	10	7	4
AUG 23	12	12	8	3
SEP 14	9	11	7	3
MEAN	11	11	7	3
SD	1	1	0	0
n	4	4	4	3
SULPHIDE (mg/L)				
AUG 3				
AUG 6				
AUG 23	< 0.01	< 0.01	< 0.01	< 0.01
SEP 14	< 0.01	< 0.01	< 0.01	< 0.01
MEAN	< 0.01	< 0.01	< 0.01	< 0.01
SD	0.00	0.00	0.00	0.00
n	2	2	2	2

APPENDIX A(1) - PHOSPHORUS

DATE (1988)	STATION			
TOTAL(ug/L)	LOWER BARBIE	MID BARBIE	LOWER FLORENCE	GOLD
AUG 3	56	41	33	
AUG 6	57	40	36	7
AUG 23	48	38	31	5
SEP 14	58	57	48	23
MEAN	55	44	37	12
SD	4	8	7	8
n	4	4	4	3
DISSOLVED(ug/L)				
AUG 3	27	19	9	
AUG 6	15	20	29	4
AUG 23	24	27	25	3
SEP 14	29	36	26	5
MEAN	24	25	22	4
SD	5	7	8	1
n	4	4	4	3
DISSOLVED ORTHO (ug/L)				
AUG 3				
AUG 6				
AUG 23				
SEP 14	12	5	5 <	2
MEAN	12	15	7 <	2
SD	0	0	0	0
n	1	1	1	1

APPENDIX A(i) - NITROGEN

DATE (1988)	STATION			
AMMONIA(ug/L)	LOWER BARBIE	MID BARBIE	LOWER FLORENCE	GOLD
AUG 3	50	31	25	
AUG 6	38	18	18	16
AUG 23	22	15	12 <	5
SEP 14	21	13	6 <	5
MEAN	33	19	15 <	9
SD	12	7	7	5
n	4	4	4	3
NITRITE(ug/L)				
AUG 3	< 5	< 5	< 5	
AUG 6	< 5	< 5	< 5	< 5
AUG 23	< 5	< 5	< 5	< 5
SEP 14	< 5	< 5	< 5	< 5
MEAN	< 5	< 5	< 5	< 5
SD	0	0	0	0
n	4	4	4	3
NITRITE+NITRATE(ug/L)				
AUG 3	10	< 7	5	
AUG 6	< 7	< 5	< 5	< 5
AUG 23	< 6	< 5	< 5	< 5
SEP 14	< 5	< 5	< 5	< 5
MEAN	< 7	< 6	< 5	< 5
SD	2	1	0	0
n	4	4	4	3

TOTAL NITROGEN(mg/L)

AUG 3	0.58	0.43	0.36	
AUG 6	0.67	0.39	0.35	0.16
AUG 23	0.54	0.43	0.38	0.15
SEP 14	0.54	0.44	0.36	0.13
MEAN	0.58	0.42	0.36	0.15
SD	0.05	0.02	0.01	0.01
n	4	4	4	3

APPENDIX A(i) - CARBON AND HUMIC CONTENT

DATE (1988)	STATION			
TOTAL ORGANIC (mg/L)	LOWER BARBIE	MID BARBIE	LOWER FLORENCE	GOLD
AUG 3	30	27	25	
AUG 6	30	29	27	6
AUG 23	28	17	25	6
SEP 14	23	27	22	3
MEAN	28	25	25	5
SD	3	5	2	1
n	4	4	4	3
TOTAL INORGANIC (mg/L)				
AUG 3	3	2	3	
AUG 6	4	2	2	3
AUG 23				
SEP 14	4	3	3	4
MEAN	4	2	3	4
SD	0	0	0	0
n	3	3	3	2
HUMIC CONTENT (Aldrich equivalent mg/L)				
AUG 3				
AUG 6	48	35	32	6
AUG 23	66	61	55	5
SEP 14	43	45	37	5
MEAN	52	47	41	5
SD	10	11	10	0
n	3	3	3	3

APPENDIX A(i) - RESIDUES AND TURBIDITY

DATE (1988)	LOWER BARBIE	MID BARBIE	LOWER FLORENCE	GOLD
NON-FILTERABLE RESIDUE(mg/L)				
AUG 3		17	19	
AUG 6	14	15 <	5 <	5
AUG 23	16	7	10 <	5
SEP 14	7	6 <	5 <	5
MEAN	12	11 <	10 <	5
SD	4	5	6	0
n	3	4	4	3
VOLATILE RESIDUE(mg/L)				
AUG 3	63	56	46	
AUG 6	59	56	41	14
AUG 23	59	63	58	12
SEP 14	70	64	49 <	5
MEAN	63	60	48 <	10
SD	4	4	6	4
n	4	4	4	3
TURBIDITY(FTU)				
AUG 3	2.8	0.8	0.3	
AUG 6	3.3	0.8	1.3	0.3
AUG 23				
SEP 14				
MEAN	3.0	0.8	0.8	0.3
SD	0	0	0	0
n	2	2	2	1

APPENDIX A(1) - TEMPERATURE AND DISSOLVED OXYGEN

DATE (1988)	STATION			
TEMPERATURE (C)	LOWER BARBIE	MID BARBIE	LOWER FLORENCE	GOLD
AUG 3	18.3	14.2	14.4	
AUG 6	14.5	12.6	12.9	16.6
AUG 23	12.9	12.5	13.5	19.0
SEP 14	13.5	12.2	12.2	16.2
MEAN	14.8	12.9	13.2	17.3
SD	2.1	0.8	0.8	1.2
n	4	4	4	3
DISSOLVED OXYGEN (mg/L)				
AUG 3	5.7	8.3	9.2	
AUG 6	5.8	9.2	8.7	8.9
AUG 23	7.0	8.1	8.4	9.3
SEP 14	9.5	8.8	12.0	11.6
MEAN	7.0	8.6	9.6	9.9
SD	1.5	0.4	1.4	1.2
n	4	4	4	3
OXYGEN SATURATION (%)				
AUG 3	62.4	83.6	93.0	
AUG 6	58.8	89.4	85.1	94.2
AUG 23	68.5	78.6	83.3	103.2
SEP 14	94.2	84.8	115.6	121.9
MEAN	71.0	84.1	94.3	106.4
SD	13.8	3.8	12.9	11.5
n	4	4	4	3

APPENDIX A(1) - HARDNESS

(mg/L CaCO3)

DATE (1988)	STATION: LOWER BARBIE Ca/Mg	LOWER BARBIE TOTAL	MID BARBIE Ca/Mg	MID BARBIE TOTAL	LOWER FLORENCE Ca/Mg	LOWER FLORENCE TOTAL	GOLD Ca/Mg	GOLD TOTAL
AUG 3	12.2	16.2	12.2	16.1	10.1	14.2		
	12.0	16.1	12.5	16.8	10.3	14.6		
	11.8	15.3	12.4	16.5	10.3	14.4		
AUG 6	12.3	16.5	13.4	18.0	10.4	14.3	13.5	14.3
	12.2	16.5	13.0	17.5	9.8	13.0	13.4	13.7
	12.4	17.5	13.2	17.8	10.3	13.3	13.7	14.3
AUG 23	13.5	19.5	13.7	19.9	11.9	16.6	15.4	16.0
	15.1	21.2	13.3	19.5	11.0	14.9	14.1	14.8
	13.0	18.1	14.5	21.2	10.9	15.4	14.1	14.6
SEP 14	12.6	18.3	13.7	20.2	11.4	16.2	14.6	15.3
	12.5	17.8	13.6	20.6	11.6	16.6	14.2	15.0
	12.3	18.4	13.6	20.5	11.7	16.2	14.1	14.7
MEAN	12.7	17.6	13.3	18.7	10.8	15.0	14.1	14.7
n	12	12	12	12	12	12	9	9

APPENDIX A - WATER QUALITY (ii) METALS

- low level mercury
- mercury and silver
- arsenic and selenium
- copper and cadmium
- lead and zinc
- calcium and magnesium
- iron and manganese
- aluminium and silica

APPENDIX A(11) - LOW LEVEL MERCURY

(ng/L)	STATION			
DATE(1988)	LOWER BARBIE	MIDDLE BARBIE	LOWER FLORENCE	GOLD
AUG 3	< 5	7	< 5	
AUG 6	31	14	< 5	< 5
AUG 23	14	15	9	6
SEP 15	12	11	8	< 5
mean	< 16	12	< 7	< 5
sd	10	3	2	0
n	4	4	4	3

APPENDIX A(11) - MERCURY AND SILVER

STATION

(ug/L)	LOWER BARBIE	MID BARBIE	LOWER FLORENCE	GOLD
DATE (1988)	THg	THg	THg	THg
AUG 3	< 0.05	< 0.05	< 0.05	< 0.05
	< 0.05	< 0.05	< 0.05	< 0.05
	< 0.05	< 0.05	< 0.05	< 0.05
AUG 6	< 0.05	< 0.05	< 0.05	< 0.05
	< 0.05	< 0.05	< 0.05	< 0.05
	< 0.05	< 0.05	< 0.05	< 0.05
AUG 23	< 0.05	< 0.05	< 0.05	< 0.05
	< 0.05	< 0.05	< 0.05	< 0.05
	< 0.05	< 0.05	< 0.05	< 0.05
SEP 14	< 0.05	< 0.05	< 0.05	< 0.05
	< 0.05	< 0.05	< 0.05	< 0.05
	< 0.05	< 0.05	< 0.05	< 0.05
MEAN	< 0.05	< 0.05	< 0.05	< 0.05
n	12	12	12	12

(ug/L)	LOWER BARBIE		MID BARBIE		LOWER FLORENCE		GOLD	
DATE (1988)	TAg	DAg	TAg	DAg	TAg	DAg	TAg	DAg
AUG 3	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
AUG 6	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
AUG 23	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
SEP 14	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
MEAN	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
n	12	12	12	12	12	12	9	9

APPENDIX A(11) - ARSENIC AND SELENIUM

STATION

(ug/L)	LOWER BARBIE		MID BARBIE		LOWER FLORENCE		GOLD	
DATE (1988)	TAs	DAs	TAs	DAs	TAs	DAs	TAs	DAs
AUG 3	8.4	2.5	3.6	< 3.3	< 0.5	< 0.5		
	8.1	2.9	4.3	3.1	< 0.6	< 0.5		
	7.9	2.2	4.3	3.5	< 0.6	< 0.5		
AUG 6	8.8	3.4	5	< 2.8	< 0.5	< 0.5	< 0.5	0.9
	7.9	2.6	3.7	< 3.3	< 0.5	< 0.5	< 0.6	< 0.5
	9.1	3.7	4.4	< 3	< 0.5	< 0.5	< 0.5	< 0.5
AUG 23	7.1	3.9	3.8	< 3.5	< 0.5	< 0.5	< 0.5	0.9
	6.6	3.1	3.1	< 3.6	< 0.5	< 0.5	0.6	0.9
	6.7	3.1	4.2	< 3.9	< 0.5	< 0.5		1.1
SEP 14								
MEAN	7.8	3.0	4.0	< 3.3	< 0.5	< 0.5	< 0.5	< 0.8
n	9	9	9	9	9	9	5	6

(ug/L)	LOWER BARBIE		MID BARBIE		LOWER FLORENCE		GOLD	
DATE (1988)	TSe	DSe	TSe	DSe	TSe	DSe	TSe	DSe
AUG 3	< 0.5	< 0.5	< 0.5	< 0.5	< 0.6	< 0.5		
	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5		
	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5		
AUG 6	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
AUG 23	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	<	0.5
SEP 14								
MEAN	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
n	9	9	9	9	9	9	5	6

APPENDIX A(11) - COPPER AND CADMIUM

STATION

(ug/L)	LOWER BARBIE		MID BARBIE		LOWER FLORENCE		GOLD	
DATE (1988)	TCu	DCu	TCu	DCu	TCu	DCu	TCu	DCu
AUG 3	0.7	0.8	0.7	0.5	0.6	0.5		
	< 0.5	< 0.5	0.8	< 0.5	1.2	< 0.5		
	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5		
AUG 6	< 0.5	< 0.5	1.3	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
	< 0.5	< 0.5	1.7	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
	0.8	< 0.5	0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
AUG 23		< 0.5		< 0.5		< 0.5		< 0.5
		< 0.5		< 0.5		< 0.5		< 0.5
		< 0.5		< 0.5		< 0.5		< 0.5
SEP 14	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
MEAN	< 0.5	< 0.5	< 0.7	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
n	9	12	9	12	9	12	6	9

(ug/L)	LOWER BARBIE		MID BARBIE		LOWER FLORENCE		GOLD	
DATE (1988)	TCd	DCd	TCd	DCd	TCd	DCd	TCd	DCd
AUG 3	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
AUG 6	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
AUG 23		< 0.1		< 0.1		< 0.1		< 0.1
		< 0.1		< 0.1		< 0.1		< 0.1
		< 0.1		< 0.1		< 0.1		< 0.1
SEP 14	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	< 0.1	< 0.1	0.1	0.2	< 0.1	< 0.1	< 0.1	< 0.1
MEAN	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
n	8	12	9	12	9	12	6	9

APPENDIX A(11) - LEAD AND ZINC

STATION

(ug/L)	LOWER BARBIE		MID BARBIE		LOWER FLORENCE		GOLD	
DATE (1988)	TPb	DPb	TPb	DPb	TPb	DPb	TPb	DPb
AUG 3	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5		
	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5		
	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5		
AUG 6	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
AUG 23		< 0.5		< 0.5		< 0.5		< 0.5
		< 0.5		< 0.5		< 0.5		< 0.5
		< 0.5		< 0.5		< 0.5		< 0.5
SEP 14	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
MEAN	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
n	9	12	9	12	9	12	6	9

(ug/L)	LOWER BARBIE		MID BARBIE		LOWER FLORENCE		GOLD	
DATE (1988)	TZn	DZn	TZn	DZn	TZn	DZn	TZn	DZn
AUG 3	2	12	3	4 <	2 <	2		
	3	3 <	2	4 <	2 <	2		
	4	3 <	2	5 <	2 <	2		
AUG 6	< 2	4 <	2	3 <	2 <	2 <	2 <	2
	< 2	< 2 <	2	3 <	2 <	2 <	2 <	2
	< 2	3 <	2	3 <	2 <	2 <	2 <	2
AUG 23		2		5	<	2	<	2
		4		13	<	2	<	2
	< 2	2		4	<	2	<	2
SEP 14	13 <	2	15	7 <	2 <	2 <	2 <	2
	9	3	14	3 <	2 <	2 <	2 <	2
	10	2 <	2	3 <	2 <	2 <	2 <	2
MEAN	< 5	< 4	< 5	5 <	2 <	2 <	2 <	2
n	9	12	9	12	9	12	6	9

APPENDIX A(ii) - CALCIUM AND MAGNESIUM

(mg/L)	STATION							
	LOWER BARBIE		MID BARBIE		LOWER FLORENCE		GOLD	
DATE (1988)	TCa	DCa	TCa	DCa	TCa	DCa	TCa	DCa
AUG 3	3.2	2.8	3	2.9	2.1	2.1		
	2.9	2.8	3.2	2.9	2.4	2.1		
	3.1	2.8	3.1	2.9	2.3	2.1		
AUG 6	3.1	2.9	3.2	3.1	2.2	2.1	4.5	4.2
	3.2	2.9	3.4	3.1	2.4	2	4.3	4.2
	3.3	2.9	3.4	3.2	2.5	2.1	4.7	4.3
AUG 23	3.5	3.2	3.5	3.3	2.6	2.5	5.2	4.9
	3.6	3.5	3.7	3.2	2.6	2.3	4.8	4.5
	3.4	3.1	3.5	3.5	2.8	2.4	5	4.5
SEP 14	3.1	2.9	3.6	3.3	2.3	2.3	4.1	4.5
	3.2	2.9	3.5	3.3	2.3	2.4	4.1	4.4
	3.2	2.9	3.3	3.2	2.3	2.4	4.1	4.4
MEAN	3.2	2.9	3.3	3.1	2.4	2.2	4.5	4.4
n	12	12	12	12	12	12	9	9

(mg/L)	STATION							
	LOWER BARBIE		MID BARBIE		LOWER FLORENCE		GOLD	
DATE (1988)	TMg	DMg	TMg	DMg	TMg	DMg	TMg	DMg
AUG 3	1.3	1.2	1.3	1.2	1.2	1.2		
	1.2	1.2	1.3	1.2	1.4	1.2		
	1.3	1.2	1.3	1.2	1.3	1.2		
AUG 6	1.3	1.2	1.3	1.3	1.3	1.2	0.8	0.7
	1.4	1.2	1.4	1.3	1.4	1.2	0.7	0.7
	1.4	1.3	1.4	1.3	1.4	1.2	0.8	0.7
AUG 23	1.5	1.3	1.5	1.3	1.5	1.3	0.8	0.8
	1.5	1.5	1.6	1.3	1.4	1.3	0.8	0.7
	1.4	1.3	1.5	1.4	1.7	1.2	0.9	0.7
SEP 14	1.2	1.3	1.3	1.3	1.3	1.4	0.8	0.8
	1.3	1.3	1.3	1.3	1.3	1.4	0.8	0.8
	1.3	1.2	1.4	1.3	1.4	1.4	0.8	0.8
MEAN	1.3	1.2	1.3	1.2	1.3	1.2	0.8	0.7
n	12	12	12	12	12	12	9	9

APPENDIX A(11) - IRON AND MANGANESE

(mg/L)	STATION							
	LOWER BARBIE		MID BARBIE		LOWER FLORENCE		GOLD	
DATE (1988)	TFe	DFe	TFe	DFe	TFe	DFe	TFe	DFe
AUG 3	5.090	1.040	2.170	0.623	1.540	0.967		
	4.740	1.090	2.290	0.709	1.720	0.982		
	5.100	0.823	2.230	0.688	1.660	0.927		
AUG 6	5.800	1.220	2.240	0.850	1.620	0.961	0.240	0.106
	5.820	1.280	2.430	0.836	1.790	0.665	0.202	0.110
	5.880	1.670	2.420	0.855	1.850	0.684	0.221	0.110
AUG 23	5.010	1.840	2.710	1.320	2.390	1.120	0.236	0.131
	5.040	1.850	2.800	1.300	2.010	0.854	0.200	0.112
	4.790	1.550	2.690	1.400	2.060	1.040	0.201	0.111
SEP 14	4.450	1.830	2.830	1.580	1.830	1.140	0.183	0.107
	4.460	1.690	2.840	1.730	1.820	1.160	0.163	0.103
	4.490	2.070	2.870	1.680	1.820	1.120	0.164	0.105
MEAN	5.056	1.496	2.543	1.131	1.843	0.968	0.201	0.111
n	12	12	12	12	12	12	9	9

(mg/L)	STATION							
	LOWER BARBIE		MID BARBIE		LOWER FLORENCE		GOLD	
DATE (1988)	TMn	DMn	TMn	DMn	TMn	DMn	TMn	DMn
AUG 3	0.203	0.152	0.137	0.126	0.027	0.020		
	0.188	0.152	0.145	0.128	0.031	0.020		
	0.202	0.147	0.142	0.128	0.029	0.019		
AUG 6	0.183	0.132	0.136	0.128	0.029	0.021	0.021	0.006
	0.185	0.130	0.143	0.126	0.032	0.015	0.020	0.007
	0.186	0.135	0.146	0.128	0.033	0.016	0.021	0.009
AUG 23	0.135	0.100	0.173	0.157	0.038	0.024	0.025	0.012
	0.136	0.108	0.177	0.156	0.037	0.020	0.023	0.011
	0.130	0.096	0.172	0.168	0.039	0.023	0.023	0.012
SEP 14	0.089	0.067	0.161	0.147	0.032	0.022	0.021	0.009
	0.089	0.065	0.162	0.146	0.031	0.022	0.021	0.008
	0.090	0.070	0.161	0.146	0.032	0.022	0.021	0.008
MEAN	0.151	0.113	0.155	0.140	0.032	0.020	0.022	0.009
n	12	12	12	12	12	12	9	9

APPENDIX A(11) - ALUMINIUM AND SILICA

(mg/L)	STATION							
	LOWER BARBIE		MID BARBIE		LOWER FLORENCE		GOLD	
DATE (1988)	TAl	DAl	TAl	DAl	TAl	DAl	TAl	DAl
AUG 3	0.70	0.34	0.61	0.45	0.49	0.41		
	0.62	0.32	0.76	0.50	0.57	0.45		
	0.67	0.31	0.66	0.47	0.54	0.42		
AUG 6	0.62	0.32	0.60	0.51	0.43	0.37	0.07	0.10
	0.69	0.30	0.73	0.49	0.46	0.34	0.06 <	0.05
	0.69	0.33	0.64	0.50	0.49	0.33	0.12	0.06
AUG 23	0.73	0.45	0.78	0.63	0.68	0.48	0.13	0.08
	0.73	0.46	0.85	0.65	0.68	0.41	0.07	0.09
	0.68	0.37	0.81	0.68	0.69	0.45	0.11 <	0.05
SEP 14	0.56	0.41	0.82	0.60	0.54	0.49	0.09	0.08
	0.61	0.39	0.78	0.63	0.51	0.51	0.10	0.09
	0.62	0.40	0.83	0.65	0.52	0.44	0.09	0.06
MEAN	0.66	0.37	0.74	0.56	0.55	0.43	0.09 <	0.07
n	12	12	12	12	12	12	9	9

(mg/L)	LOWER BARBIE		MID BARBIE		LOWER FLORENCE		GOLD	
DATE (1988)	TSi	DSi	TSi	DSi	TSi	DSi	TSi	DSi
AUG 3	2.41	2.24	3.08	3.13	4.41	4.50		
	2.25	2.24	3.26	3.14	4.88	4.64		
	2.40	2.23	3.17	3.13	4.71	4.64		
AUG 6	2.47	2.35	3.26	3.33	4.69	4.79	1.28	1.20
	2.49	2.32	3.50	3.27	5.10	4.76	1.25	1.17
	2.52	2.33	3.48	3.38	5.27	4.88	1.35	1.20
AUG 23	2.47	2.46	3.04	3.07	4.33	4.59	1.22	1.20
	2.49	2.62	3.16	3.11	4.37	4.28	1.16	1.16
	2.35	2.43	3.02	3.29	4.37	4.26	1.16	1.15
SEP 14	2.08	2.44	2.83	3.36	4.14	4.74	1.10	1.24
	2.09	2.41	2.82	3.35	4.16	4.78	1.11	1.22
	2.07	2.40	3.15	3.40	4.24	4.78	1.10	1.20
MEAN	2.34	2.37	3.15	3.25	4.56	4.64	1.19	1.19
n	12	12	12	12	12	12	9	9

APPENDIX A (iii) STREAM VELOCITY

APPENDIX A(iii) - STREAM VELOCITY

(cm/s)	STATION		
DATE(1988)	LOWER BARBIE	MIDDLE BARBIE	GOLD
AUG 6	1-2	2-3	4
AUG 8	4	5-9	5-6
AUG 23	3	5	4-5
SEP 16	3	3	11

APPENDIX A (iv) PALLANT HATCHERY

APPENDIX A - WATER QUALITY (iv) PALLANT HATCHERY
(SEPTEMBER 15 1988)

IMMEDIATES	
pH	7.1
ACIDITY(mg/L CaCO3)	1.7
ALKALINITY(mg/L CaCO3)	16.5
HARDNESS(mg/L CaCO3)	18.9
CHLORIDE(mg/L)	2.8
SULPHATE(mg/L)	2
AMMONIA*(ug/L)	<5
NITRITE(ug/L)	<5
NITRITE/NITRATE(ug/L)	18
TOTAL ORGANIC CARBON(mg/L)	<1
NON-FILTERABLE RESIDUE(mg/L)	<5

METALS	TOTAL	DISSOLVED
MERCURY(ug/L)	<0.05	
ARSENIC(ug/L)		
SELENIUM(ug/L)		
COPPER(ug/L)	<0.5	<0.5
CADMIUM(ug/L)	<0.1	<0.1
LEAD(ug/L)	<0.5	<0.5
ZINC(ug/L)	<2	<2
CALCIUM(mg/L)	5.5	6.0
IRON(mg/L)	0.073	0.041
MAGNESIUM(mg/L)	1.0	0.9
MANGANESE(mg/L)	0.008	0.003

APPENDIX B - SEDIMENT QUALITY (i) NON METALS

- redox and heterotrophic bacteria
- total phosphorus and total nitrogen
- sediment volatile residue
- total Kjeldahl nitrogen, total sulfur and sulfate sulfur

APPENDIX B(1) - REDOX AND HETEROTROPHIC BACTERIA

	REDOX (mv)		
(1988)	LOWER BARBIE	MID BARBIE	GOLD
AUG 3-6	+200	+420	+140
	+75	+330	+170
AUG 22-24	+240	+360	+200
	-70	+370	-30
	+60	+390	+130
SEP 14-16	+50	+230	+300
	-20	+350	0
	+100	+70	+70
median	+60	+350	+130

	HETEROTROPHIC BACTERIA (CFU/g)		
(1988)	LOWER BARBIE	MID BARBIE	GOLD
AUG 3	67000	31000	176000
AUG 24	119000	39000	410000
SEP 15	110000	118000	141000

APPENDIX B(1) - TOTAL PHOSPHORUS AND TOTAL NITROGEN (<0.15mm)

(mg/g)	STATION					
	LOWER BARBIE		MID BARBIE		GOLD	
DATE (1988)	TP	TN	TP	TN	TP	TN
AUG 6	1.10	5.6	0.76	2.1	1.00	6.7
	1.10	4.9	0.85	2.2	0.90	7.3
	1.00	7.5	0.83	1.8	0.85	4.9
	1.10	7.9	0.93	1.9	0.81	5.5
	1.10	6.6	0.80	1.4	0.85	6.4
MEAN	1.08	6.5	0.83	1.9	0.88	6.2
SD	0.04	1.1	0.06	0.3	0.07	0.9
n	5	5	5	5	5	5
SEP 16	1.00	5.6	0.80	2.1	0.87	6.7
	0.92	4.9	0.98	2.2	0.80	7.3
	0.91	7.5	0.95	1.8	1.30	4.9
	1.10	7.9	0.94	1.9	0.79	5.5
	1.10	6.6	1.00	1.4	1.00	6.4
MEAN	1.01	6.5	0.93	1.9	0.95	6.2
SD	0.08	1.1	0.07	0.3	0.19	0.9
n	5	5	5	5	5	5
OVERALL MEAN	1.04	6.5	0.88	1.9	0.92	6.2
SD	0.07	1.1	0.08	0.3	0.15	0.9
n	10	10	10	10	10	10

APPENDIX B(i) - SEDIMENT VOLATILE RESIDUE (<0.15mm)

(%)	STATION		
	LOWER BARBIE	MID BARBIE	GOLD
DATE (1988)	SVR	SVR	SVR
AUG 6	23.0	15.6	21.1
	27.2	13.5	25.9
	27.0	13.3	20.7
	32.8	15.3	22.7
	29.8	9.9	25.1
MEAN	28.0	13.5	23.1
SD	3.3	2.0	2.1
n	5	5	5
SEP 16	27.2	12.4	21.0
	26.8	17.4	33.4
	28.5	19.0	22.6
	28.9	10.6	25.0
	33.7	11.4	22.5
MEAN	29.0	14.2	24.9
SD	2.5	3.4	4.4
n	5	5	5
OVERALL MEAN	28.5	13.8	24.0
SD	2.9	2.8	3.6
n	10	10	10

APPENDIX B(1) - TOTAL KJELDAHL NITROGEN, TOTAL SULFUR AND SULFATE SULFUR
(whole sample)

(mg/g)	STATION				
	LOWER BARBIE		MIDDLE BARBIE		GOLD
TOTAL KJELDAHL NITROGEN					
AUG 6 1988	6.06		0.64		1.69
SEP 16 1988	5.17		0.56		3.23
TOTAL SULFUR					
AUG 6 1988	1.21		0.10		0.55
SEP 16 1988	3.63		0.10		1.07
SULFATE SULFUR					
AUG 6 1988	0.5	<	0.1		0.2
SEP 16 1988	0.8	<	0.1		0.5
SULFIDE SULFUR*					
AUG 6 1988	1.04	<	0.07		0.48
SEP 16 1988	3.36	<	0.07		0.90

* calculated (TOTAL SULFUR - SULFIDE SULFUR)

APPENDIX B - SEDIMENT QUALITY (ii) METALS

- arsenic and mercury
- cadmium and copper
- lead and zinc
- chromium and nickel
- barium and vanadium
- aluminium and iron
- magnesium and manganese
- calcium and silica

APPENDIX B(11) - ARSENIC AND MERCURY (<0.15mm)

(ug/g)	STATION					
	LOWER BARBIE		MID BARBIE		GOLD	
DATE (1988)	As	Hg	As	Hg	As	Hg
AUG 6	63	0.23	71	0.09	97	0.15
	53	0.29	82	0.09	92	0.13
	53	0.30	78	0.09	72	0.13
	87	0.29	97	0.09	110	0.14
	82	0.28	83	0.09	110	0.15
MEAN	68	0.28	82	0.09	96	0.14
SD	14	0.02	9	0.00	14	0.01
n	5	5	5	5	5	5
SEP 16	63	0.24	25	0.13	9	0.20
	53	0.22	61	0.12	26	0.12
	53	0.22	49	0.12 <	8	0.22
	87	0.25	62	0.14	27	0.13
	82	0.25	71	0.10 <	8	0.15
MEAN	68	0.24	54	0.12 <	16	0.16
SD	14	0.01	16	0.01	9	0.04
n	5	5	5	5	5	5
OVERALL MEAN	68	0.26	68	0.11 <	56	0.15
SD	14	0.03	19	0.02	42	0.03
n	10	10	10	10	10	10

APPENDIX B(11) - CADMIUM AND COPPER (<.15mm)

(ug/g)		STATION					
		LOWER BARBIE		MID BARBIE		GOLD	
DATE (1988)		Cd	Cu	Cd	Cu	Cd	Cu
AUG 6		1.0	16.0	1.0	16.2	1.8	17.7
		1.0	16.9	2.2	18.7	1.8	19.4
		0.9	16.5	1.9	18.0	1.8	17.0
		1.9	18.3	1.3	20.4 <	0.8	16.0
		1.8	17.7	2.0	18.3	1.8	17.3
MEAN		1.3	17.1	1.7	18.3	1.6	17.5
SD		0.4	0.8	0.5	1.3	0.4	1.1
n		5	5	5	5	5	5
SEP 16		1.8	18.7 <	0.8	15.0 <	0.8	12.0
		1.0	15.8 <	0.8	16.3 <	0.8	20.1
		2.0	18.7 <	0.8	16.3 <	0.8	15.0
		1.9	18.8 <	0.8	15.0 <	0.8	16.0
	<	0.8	14.0 <	0.8	17.0 <	0.8	13.0
MEAN		1.5	17.2 <	0.8	15.9 <	0.8	15.2
SD		0.5	2.0	0.0	0.8	0.0	2.8
n		5	5	5	5	5	5
OVERALL MEAN	<	1.4	17.1 <	1.2	17.1 <	1.2	16.4
SD		0.5	1.5	0.5	1.6	0.5	2.4
n		10	10	10	10	10	10

APPENDIX B(11) - LEAD AND ZINC (<0.15mm)

(ug/g)		STATION					
		LOWER BARBIE		MID BARBIE		GOLD	
DATE (1988)		Pb	Zn	Pb	Zn	Pb	Zn
AUG 6		21	224 <	8	115	20	165
		22	273	10	130	20	216
		18	231	10	140	10	157
		10	265	21	122	20	148
		20	236	21	134	20	136
MEAN		18	246	14	128	18	164
SD		4	19	6	9	4	28
n		5	5	5	5	5	5
SEP 16		10	181 <	8	120 <	8	56.9
	<	8	173 <	8	132 <	8	70.2
		25	200 <	8	115 <	8	66.8
		25	196 <	8	104 <	8	84.7
	<	8	196 <	8	107 <	8	61.6
MEAN	<	15	189 <	8	116 <	8	68.0
SD		8	10	0	10	0	9.5
n		5	5	5	5	5	5
OVERALL MEAN	<	17	217 <	11	122 <	13	116
SD		7	32	5	11	6	52
n		10	10	10	10	10	10

APPENDIX B(ii) - CHROMIUM AND NICKEL (<0.15mm)

(ug/g)	STATION					
	LOWER BARBIE		MID BARBIE		GOLD	
DATE (1988)	Cr	Ni	Cr	Ni	Cr	Ni
AUG 6	21.4	10	19.8	7	11.0 <	3
	24.7	10	22.8	8	11.0 <	3
	22.4	10	22.9	8	11.0 <	3
	22.5	10	25.2	9	8.6 <	3
	23.4	10	23.6	10	10.0 <	3
MEAN	22.9	10	22.9	8	10.3 <	3
SD	1.1	0	1.9	1	0.9	0
n	5	5	5	5	5	5
SEP 16	22.2	8	23.9	10	10.0	4
	20.3	6	25.1	10	11.0	4
	21.3	10	24.6	10	15.0	4
	22.9	10	24.0	8	11.0 <	3
	21.2	10	25.1	10	11.0	3
MEAN	21.6	9	24.5	10	11.6 <	4
SD	0.9	2	0.5	1	1.7	0
n	5	5	5	5	5	5
OVERALL MEAN	22.2	9	23.7	9	11.0	3
SD	1.2	1	1.5	1	1.5	0
n	10	10	10	10	10	10

APPENDIX B(ii) - BARIUM AND VANADIUM (<0.15mm)

(ug/g)	STATION					
	LOWER BARBIE		MID BARBIE		GOLD	
DATE (1988)	Ba	V	Ba	V	Ba	V
AUG 6	146	93	95.8	75	53.4	83
	173	96	98.3	86	59.8	75
	170	89	98.8	87	43.9	65
	193	85	99.7	96	38.7	78
	188	94	91.1	87	42.0	87
MEAN	174	91	96.7	86	47.6	78
SD	16	4	3.1	7	7.8	8
n	5	5	5	5	5	5
SEP 16	142	88	112	84	45.9	65
	146	82	135	99	63.5	65
	147	90	127	95	51.6	100
	158	96	114	93	60.9	73
	184	88	115	96	49.2	60
MEAN	155	89	121	93	54.2	73
SD	15	4	9	5	6.8	14
n	5	5	5	5	5	5
OVERALL MEAN	165	90	109	90	50.9	75
SD	18	4	14	7	8.1	12
n	10	10	10	10	10	10

APPENDIX B(11) - ALUMINIUM AND IRON (<0.15mm)

(mg/g)	STATION					
	LOWER BARBIE		MID BARBIE		GOLD	
DATE (1988)	Al	Fe	Al	Fe	Al	Fe
AUG 6	23.1	37.4	17.1	39.0	23.0	51.6
	26.2	33.7	19.8	45.6	19.7	61.2
	23.8	32.1	19.1	46.3	18.6	38.2
	23.6	42.8	19.7	53.0	19.6	49.8
	24.8	38.4	18.0	44.6	20.4	47.3
MEAN	24.3	36.9	18.7	45.7	20.3	49.6
SD	1.1	3.8	1.0	4.5	1.5	7.4
n	5	5	5	5	5	5
SEP 16	23.7	32.9	29.8	39.9	26.5	21.6
	23.2	35.4	31.4	47.7	28.5	31.6
	22.3	45.2	32.5	48.2	43.4	20.9
	23.7	39.8	29.2	47.8	29.4	34.1
	35.6	38.6	30.2	52.1	28.6	19.0
MEAN	25.7	38.4	30.6	47.1	31.3	25.4
SD	5.0	4.2	1.2	4.0	6.1	6.2
n	5	5	5	5	5	5
OVERALL MEAN	25.0	37.6	24.7	46.4	25.8	37.5
SD	3.7	4.0	6.0	4.3	7.1	13.9
n	10	10	10	10	10	10

APPENDIX B(11) - MAGNESIUM AND MANGANESE (<0.15mm)

(mg/g)	STATION					
	LOWER BARBIE		MID BARBIE		GOLD	
DATE (1988)	Mg	Mn	Mg	Mn	Mg	Mn
AUG 6	3.55	4.57	3.84	1.46	2.42	0.84
	4.11	3.01	4.48	1.09	2.61	0.96
	3.75	4.72	4.39	1.16	2.87	1.30
	3.61	5.16	4.48	1.46	1.93	1.97
	3.78	5.19	4.37	0.91	2.30	1.86
MEAN	3.76	4.53	4.31	1.22	2.43	1.39
SD	0.19	0.80	0.24	0.22	0.31	0.46
n	5	5	5	5	5	5
SEP 16	3.87	1.71	6.42	0.85	3.90	0.49
	3.71	2.69	6.50	3.60	3.80	0.74
	3.37	2.96	6.56	1.17	4.12	0.76
	3.48	2.65	6.59	1.63	4.07	1.14
	5.20	3.98	6.77	1.18	3.88	0.58
MEAN	3.93	2.80	6.57	1.69	3.95	0.74
SD	0.66	0.73	0.12	0.99	0.12	0.22
n	5	5	5	5	5	5
OVERALL MEAN	3.84	3.66	5.44	1.45	3.19	1.06
SD	0.49	1.15	1.14	0.75	0.80	0.48
n	10	10	10	10	10	10

APPENDIX B(ii) - CALCIUM AND SILICA (<0.15mm)

(mg/g)	STATION					
	LOWER BARBIE		MID BARBIE		GOLD	
DATE (1988)	Ca	Si	Ca	Si	Ca	Si
AUG 6	3.70	1.41	3.07	1.21	4.16	0.65
	4.42	1.29	3.01	1.39	4.43	0.58
	4.28	1.34	3.03	1.29	4.66	0.70
	4.91	1.28	3.01	1.37	4.02	0.63
	4.50	1.31	2.91	1.19	4.06	0.64
MEAN	4.36	1.33	3.01	1.29	4.27	0.64
SD	0.39	0.05	0.05	0.08	0.24	0.04
n	5	5	5	5	5	5
SEP 16	3.83	1.07	2.95	1.26	3.70	0.67
	3.42	1.13	3.31	1.18	4.83	0.57
	4.55	1.14	2.97	1.23	3.43	1.16
	4.10	1.05	2.68	1.30	3.67	0.64
	4.13	1.12	2.64	1.20	3.33	0.68
MEAN	4.01	1.10	2.91	1.23	3.79	0.75
SD	0.37	0.04	0.24	0.04	0.54	0.21
n	5	5	5	5	5	5
OVERALL MEAN	4.18	1.21	2.96	1.26	4.03	0.69
SD	0.42	0.12	0.18	0.07	0.48	0.16
n	10	10	10	10	10	10

APPENDIX B - SEDIMENT QUALITY (iii) REFERENCE SEDIMENT

APPENDIX B(iii): - REFERENCE SEDIMENT

AUGUST 6 1988 SAMPLES

METAL (ug/g)	WEST VANCOUVER LABORATORY (i)	(ii)	(iii)	mean	sd	rsd (%)
MERCURY	0.182	0.201	0.188	0.190	0.008	4.2
ARSENIC	22	23	23	23	0	
CADMIUM	1.7	1.0	1.7	1.5	0.3	22.5
CHROMIUM	31.5	32.6	32.1	32.1	0.4	1.4
COPPER	23.2	22.3	23.5	23.0	0.5	2.2
LEAD	34	35	38	36	2	4.8
MANGANESE	374	372	366	371	3	0.9
NICKEL	20	20	21	20	0	
VANADIUM	41	40	41	41	0	
ZINC	199	200	192	197	4	1.8

METAL	MESS-1 CERTIFIED VALUE (+-95%limits)	WEST VANCOUVER LABORATORY (MEAN % RECOVERY)*
MERCURY	.171 (.157-.185)	111
ARSENIC	10.6 (9.4-11.8)	
CADMIUM	.59 (.49-.69)	
CHROMIUM	71 (60-82)	
COPPER	25.1 (21.3-28.9)	92
LEAD	34.0 (27.9-40.1)	
MANGANESE	513 (488-538)	
NICKEL	29.5 (26.8-32.2)	
VANADIUM	72.4 (67.1-77.7)	
ZINC	191 (174-208)	103

* based on mean certified value

APPENDIX B(iii) - REFERENCE SEDIMENT

SEPTEMBER 16 1988 SAMPLES

METAL (ug/g)	WEST VANCOUVER LABORATORY (i)	(ii)	(iii)	mean	sd	rsd (%)
MERCURY	0.192	0.189	0.183	0.188	0.004	2.0
ARSENIC	<8	<8	<9	<8	0	
CADMIUM	<.8	<.8	1.0	<.9	0.1	
CHROMIUM	30.3	30.0	31.1	30.5	0.5	1.5
COPPER	23.8	21.4	26.2	23.8	2.0	8.2
LEAD	29	24	30	28	3	9.5
MANGANESE	352	345	350	349	3	0.8
NICKEL	20	18	21	20	1	
VANADIUM	39	36	36	37	1	
ZINC	185	176	178	180	4	2.1

	MESS-1 CERTIFIED VALUE (+-95%limits)	WEST VANCOUVER LABORATORY (MEAN % RECOVERY)*
MERCURY	.171 (.157-.185)	110
ARSENIC	10.6 (9.4-11.8)	
CADMIUM	.59 (.49-.69)	
CHROMIUM	71 (60-82)	
COPPER	25.1 (21.3-28.9)	95
LEAD	34.0 (27.9-40.1)	
MANGANESE	513 (488-538)	
NICKEL	29.5 (26.8-32.2)	
VANADIUM	72.4 (67.1-77.7)	
ZINC	191 (174-208)	94

* based on mean certified value

APPENDIX C - FISH TISSUE MERCURY (i) CAGED FISH

- mercury levels
- weight, length, and condition factor

APPENDIX C(1) - MERCURY LEVELS

STATION

MERCURY (ug/g)	PALLANT HATCHERY DAY-0		LOWER BARBIE DAY-42		MIDDLE BARBIE DAY-42		GOLD DAY-42	
	DRY WT	WET WT	DRY WT	WET WT	DRY WT	WET WT	DRY WT	WET WT
#1	0.242	0.052	0.260	0.053	0.282	0.056	0.286	0.059
#2	0.256	0.053	0.318	0.065	0.282	0.057	0.297	0.063
#3	0.252	0.056	0.331	0.065	0.291	0.057	0.309	0.063
#4	0.260	0.056	0.326	0.067	0.281	0.058	0.304	0.065
#5	0.273	0.056	0.336	0.067	0.294	0.059	0.322	0.065
#6	0.270	0.058	0.343	0.067	0.304	0.061	0.343	0.069
#7	0.287	0.061	0.346	0.070	0.305	0.063	0.343	0.071
#8	0.294	0.065	0.350	0.071	0.345	0.070	0.395	0.076
mean	0.267	0.057	0.326	0.066	0.298	0.060	0.325	0.066
sd	0.017	0.004	0.027	0.005	0.020	0.004	0.033	0.005
n	8	8	8	8	8	8	8	8

MOISTURE (%)	PALLANT HATCHERY DAY-0	LOWER BARBIE DAY-42	MIDDLE BARBIE DAY-42	GOLD DAY-42
	MOISTURE	MOISTURE	MOISTURE	MOISTURE
#1	78.5	79.8	80.0	79.5
#2	79.3	79.6	79.9	78.8
#3	77.8	80.3	80.3	79.7
#4	78.4	79.3	79.4	78.7
#5	79.6	80.0	79.9	79.7
#6	78.7	80.6	79.9	79.9
#7	78.8	79.9	79.4	79.4
#8	77.9	79.7	79.6	80.7
mean	78.6	79.9	79.8	79.6
sd	0.6	0.4	0.3	0.6
n	8	8	8	8

APPENDIX C(i) - WEIGHT, LENGTH, AND CONDITION FACTOR

	STATION					
	PALLANT HATCHERY DAY-0			LOWER BARBIE DAY-42		
	length (cm)	weight (g)	condition factor	length (cm)	weight (g)	condition factor
#1	10.2	13.7	1.3	9.8	10.7	1.1
#2	9.8	11.0	1.2	9.9	11.5	1.2
#3	10.2	14.6	1.4	10.4	11.8	1.0
#4	11.0	16.7	1.3	10.2	11.8	1.1
#5	10.2	13.5	1.3	10.6	12.4	1.0
#6	11.0	16.7	1.3	9.6	9.7	1.1
#7	10.2	13.8	1.3	10.4	12.4	1.1
#8	9.8	11.0	1.2	10.1	10.8	1.0
mean	10.3	13.9	1.3	10.1	11.4	1.1
sd	0.4	2.0	0.1	0.3	0.9	0.0
n	8	8	8	8	8	8

	MIDDLE BARBIE DAY-42			GOLD DAY-42		
	length (cm)	weight (g)	condition factor	length (cm)	weight (g)	condition factor
#1	10.2	13.0	1.2	9.5	11.4	1.3
#2	9.7	10.1	1.1	11.0	16.7	1.3
#3	9.8	11.8	1.3	9.5	9.6	1.1
#4	10.2	12.7	1.2	9.1	9.9	1.3
#5	10.4	12.7	1.1	9.0	7.9	1.1
#6	10.8	15.3	1.2	10.1	12.3	1.2
#7	10.2	13.5	1.3	9.7	11.4	1.2
#8	10.0	11.9	1.2	8.1	6.0	1.1
mean	10.2	12.6	1.2	9.5	10.6	1.2
sd	0.3	1.4	0.1	0.8	3.0	0.1
n	8	8	8	8	8	8

APPENDIX C - MUSCLE TISSUE MECURY (ii) FERAL FISH

- mercury levels
- weight, length, and condition factor

APPENDIX C(ii) - MERCURY LEVELS

STATION

MERCURY (ug/g)	LOWER BARBIE (AUG 17 1988)		LOWER BARBIE (SEP 15 1988)		MIDDLE BARBIE (AUG 17 1988)		GOLD (SEP 15 1988)	
	DRY WT	WET WT	DRY WT	WET WT	DRY WT	WET WT	DRY WT	WET WT
#1		0.145		0.123		0.146		0.074
#2		0.150		0.140		0.166		0.091
#3		0.167		0.154		0.172		0.095
#4		0.197		0.157		0.177		0.096
#5		0.199		0.172		0.186		0.117
#6		0.208		0.180		0.193		0.132
#7		0.216		0.198		0.197		0.209
#8		0.260		0.241		0.238		0.256
mean		0.193		0.171		0.184		0.134
sd		0.036		0.034		0.025		0.060
n		8		8		8		8

MOISTURE (%)	LOWER BARBIE (AUG 17 1988)	LOWER BARBIE (SEP 15 1988)	MIDDLE BARBIE (AUG 17 1988)	GOLD (SEP 15 1988)
	MOISTURE	MOISTURE	MOISTURE	MOISTURE
#1	79.5	80.2	81.1	79.9
#2	79.8	79.6	80.1	81.1
#3	79.5	80.1	80.5	80.3
#4	80.1	80.1	81.0	80.4
#5	80.1	79.6	80.2	78.4
#6	80.6	79.8	81.3	79.1
#7	80.0	81.0	80.5	80.3
#8	81.0	79.9	80.8	81.7
mean	80.1	80.0	80.7	80.2
sd	0.5	0.4	0.4	1.0
n	8	8	8	8

APPENDIX C(ii) - WEIGHT, LENGTH, AND CONDITION FACTOR

STATION	LOWER BARBIE (AUG 17 1988)			LOWER BARBIE (SEP 15 1988)		
	length (cm)	weight (g)	condition factor	length (cm)	weight (g)	condition factor
#1	7.5	6.5	1.5	7.7	7.7	1.7
	9.0	10.8	1.5	7.5	6.5	1.5
#2	7.4	8.3	2.0	9.3	13.3	1.7
	7.5	7.7	1.8	8.1	8.3	1.6
#3	9.1	9.2	1.2	8.1	9.0	1.7
	8.3	7.7	1.3	8.1	8.8	1.7
#4	8.3	10.4	1.8	9.0	11.0	1.5
	8.2	10.0	1.8	7.8	7.3	1.5
#5	8.4	8.0	1.3	8.2	10.9	2.0
	7.9	6.0	1.2	7.6	7.2	1.6
#6	9.0	8.3	1.1	7.7	8.0	1.8
	8.0	6.5	1.3	8.1	10.0	1.9
#7	8.2	7.0	1.3	8.1	11.4	2.1
	8.1	7.5	1.4	8.7	10.9	1.7
#8	8.2	7.0	1.3	7.6	7.4	1.7
	8.2	6.5	1.2	7.4	6.6	1.6
mean	8.2	8.0	1.4	8.1	9.0	1.7
sd	0.5	1.4	0.3	0.5	1.9	0.2
n	16	16	16	16	16	16

STATION	MID BARBIE (AUG 17 1988)			GOLD (SEP 15 1988)		
	length (cm)	weight (g)	condition factor	length (cm)	weight (g)	condition factor
#1	7.2	5.5	1.5	8.2	10.8	2.0
	7.9	9.7	2.0	8.6	10.2	1.6
#2	7.8	8.1	1.7	8.6	8.2	1.3
	9.0	9.5	1.3	10.1	11.5	1.1
#3	8.0	7.6	1.5	8.7	9.5	1.4
	7.6	6.6	1.5	9.4	9.2	1.1
#4	8.7	8.4	1.3	9.1	9.1	1.2
	8.0	10.6	2.1	10.9	13.8	1.1
#5	8.3	9.9	1.7	9.5	12.9	1.5
	8.1	8.5	1.6	7.2	6.4	1.7
#6	8.8	8.4	1.2	9.6	14.1	1.6
	8.6	7.7	1.2	8.0	8.6	1.7
#7	8.4	7.7	1.3	9.7	11.3	1.2
	8.5	7.0	1.1	9.6	9.4	1.1
#8	8.8	8.4	1.2	8.0	6.9	1.3
	8.6	7.7	1.2	9.6	8.2	0.9
mean	8.3	8.2	1.5	9.1	10.0	1.4
sd	0.5	1.3	0.3	0.9	2.2	0.3
n	16	16	16	16	16	16

APPENDIX C - MUSCLE TISSUE MERCURY (iii) REFERENCE TISSUE

APPENDIX C(iii) REFERENCE TISSUE

METAL (ug/g)	TORT 1 MEAN CERTIFIED VALUE (+-95%limits)	WEST VANCOUVER LABORATORY (i)	(ii)	(iii)	mean	sd	rsd (%)
MERCURY	0.33 (0.27-0.39)						
-initial hatchery and caged		0.200	0.175	0.198	0.191	0.011	6
-feral		0.319	0.301	0.281	0.300	0.016	5
-hatchery repeat		0.288	0.274	0.270	0.277	0.008	3

	TUNA 50 MEAN CERTIFIED VALUE (+-95%limits)	WEST VANCOUVER LABORATORY (i)	(ii)	(iii)	mean	sd	rsd (%)
MERCURY	0.95 (0.85-1.05)						
-initial hatchery and caged		0.699	0.779	0.769	0.749	0.036	5
-feral		0.801	0.840	0.873	0.838	0.029	4
-hatchery repeat		0.841	0.844	0.856	0.847	0.006	1

OTHER METALS* (ug/g)	TORT 1 MEAN CERTIFIED VALUE (+-95%limits)	WEST VANCOUVER LABORATORY (i)	(ii)	(iii)	mean	sd	rsd (%)
ARSENIC	24.6 (22.4-26.8)	28	25	20	24	3	14
CADMIUM	26.3 (24.2-28.4)	23.5	24.4	24.2	24.0	0.4	2
CHROMIUM	2.4 (1.8-3.0)	1.6	1.7	1.7	1.7	0.0	
COPPER	439 (417-461)	376	387	393	385	7	2
LEAD	10.4 (8.4-12.4)	9.5	14.0	9.6	11.0	2.1	19
ZINC	177 (167-187)	149	154	155	153	3	2

* from initial hatchery and caged reference tissue analysis

APPENDIX C(iv) - MERCURY LEVELS

STATION

MERCURY (ug/g)	AUG 5 1988 PALLANT HATCHERY DAY-0 DRY WT	AUG 5 1988 PALLANT HATCHERY DAY-0 WET WT	AUG 5 1988* PALLANT HATCHERY DAY-0 DRY WT	AUG 5 1988* PALLANT HATCHERY DAY-0 WET WT	SEP 15 1988 PALLANT HATCHERY DAY -42 DRY WT	SEP 15 1988 PALLANT HATCHERY DAY -42 WET WT
#1	0.052		0.050		0.048	
#2	0.053		0.052		0.051	
#3	0.056		0.053		0.052	
#4	0.056		0.055		0.056	
#5	0.056		0.055		0.057	
#6	0.058		0.062		0.057	
#7	0.061		0.063		0.058	
#8	0.065		0.069		0.069	
mean	0.057		0.057		0.056	
sd	0.004		0.006		0.006	
n	8		8		8	

* DAY-0 sample retested with SEP 15 1988, DAY-42 sample

MOISTURE (%)	PALLANT HATCHERY DAY-0 MOISTURE	LOWER BARBIE DAY-42 MOISTURE	MIDDLE BARBIE DAY-42 MOISTURE
#1	78.5	76.7	79.3
#2	79.3	77.6	78.0
#3	77.8	76.4	78.0
#4	78.4	76.8	78.6
#5	79.6	76.5	77.3
#6	78.7	77.9	78.6
#7	78.8	78.4	78.6
#8	77.9	75.9	77.9
mean	78.6	77.0	78.3
sd	0.6	0.8	0.6
n	8	8	8

APPENDIX C(iv) - WEIGHT, LENGTH, AND CONDITION FACTOR

	STATION					
	PALLANT HATCHERY DAY-0			PALLANT HATCHERY DAY-0*		
	length (cm)	weight (g)	condition factor	length (cm)	weight (g)	condition factor
#1	10.2	13.7	1.3	9.2	15.6	2.0
#2	9.8	11.0	1.2	8.2	10.8	2.0
#3	10.2	14.6	1.4	8.9	11.5	1.6
#4	11.0	16.7	1.3	9.4	14.1	1.7
#5	10.2	13.5	1.3	8.7	11.8	1.8
#6	11.0	16.7	1.3	8.2	10.1	1.8
#7	10.2	13.8	1.3	8.4	10.1	1.7
#8	9.8	11.0	1.2	8.9	13.5	1.9
mean	10.3	13.9	1.3	8.7	12.2	1.8
sd	0.4	2.0	0.1	0.4	1.9	0.1
n	8	8	8	8	8	8

	PALLANT HATCHERY DAY-42		
	length (cm)	weight (g)	condition factor
#1	10.6	15.8	1.3
#2	10.6	17.2	1.4
#3	11.5	19.9	1.3
#4	9.8	12.4	1.3
#5	11.6	23.4	1.5
#6	10.9	17.0	1.3
#7	10.3	15.0	1.4
#8	11.2	20.4	1.5
mean	10.8	17.6	1.4
sd	0.6	3.2	0.1
n	8	8	8

APPENDIX D - FISH RATION QUALITY

APPENDIX D - FISH RATION QUALITY

METALS		PALLANT HATCHERY*		CAGE STUDY**	
		DRY WT	WET WT	DRY WT	WET WT
MERCURY	(ug/g)	0.072	0.049	.045	0.037
CADMIUM	(ug/g)	<0.4		<0.4	
COPPER	(ug/g)	13.8	9.4	11.8	9.7
IRON	(ug/g)	218	149	597	492
LEAD	(ug/g)	<4		<4	
ZINC	(ug/g)	211	144	147	121
CALCIUM	(mg/g)	18.7		19.7	
MOISTURE	(%)	31.8		17.5	

MANUFACTURER'S ANALYSIS			
CRUDE PROTEIN	(%)	>35	>36
CRUDE FAT	(%)	>10	>13.5
CRUDE FIBRE	(%)	<4	<2
MOISTURE	(%)	<35	<23

* Formula II Oregon Moist (3.2mm)
** Biodiet (1.3mm)

APPENDIX E - FISH TRANSPORTATION

- dissolved oxygen and temperature

APPENDIX E - FISH TRANSPORTATION
(dissolved oxygen and temperature)

AUGUST 5 1988	TEMPERATURE(C)		DISSOLVED OXYGEN(mg/L)		OXYGEN SATURATION(%)	
CONTAINER	START	END	START	END	START	END
1	12.5	11.9	12.7	8.6	123	82
2	12.5	11.8	13.2	7.5	128	72
3	12.3	11.2	15.4	8.4	149	79
4	13.3	12.0	10.5	7.8	104	75

SEPTEMBER 15 1988 - AMBIENT CONDITIONS

CONTAINER	TEMPERATURE(C)	DISSOLVED OXYGEN(mg/L)	OXYGEN SATURATION(%)
1(start)	15.2	13.4	138
2(start)	15.2	12.8	132
1(2hr.)	15.2	8.2	84
2(2hr.)	15.2	7.3	75
1(5hr.)	15.5	9.2	95
2(5hr.)	15.5	8.3	86

SEPTEMBER 15 1988 - HEATED CONDITIONS

CONTAINER	TEMPERATURE(C)	DISSOLVED OXYGEN(mg/L)	OXYGEN SATURATION(%)
1(start)	15.2	14.0	144
2(start)	15.2	13.6	140
1(2hr.)	17.5	6.8	73
2(2hr.)	17.5	6.2	67
1(5hr.)	17.5	6.8	73
2(5hr.)	17.8	6.2	67

APPENDIX F - CBR INTERNATIONAL (i) FINAL REPORT

Our Reference Number: CBR 135

DSS File Number: 13SB.KA601-8-3359

June 15, 1989

Final Report
Results and Interpretation of Analytical Services Relating to an Emplacement Study Using Juvenile Coho Salmon in the Queen Charlotte Islands

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1.0 INTRODUCTION

An environmental baseline emplacement study was conducted in the vicinity of a proposed mine site on Graham Island in the Queen Charlotte Islands. The study was initially proposed to assess the effects on fish of potentially elevated environmental metals levels resulting from mining activity.

Test specimen fish, juvenile coho hatchery salmon, were caged at three selected stream sites for a period of six weeks. Prior to and at the conclusion of the emplacement study, samples of hatchery fish and also specimens of feral coho from each stream were obtained for reference and comparative purposes, respectively. Reference, emplaced and feral fish and stream water samples were submitted for analysis.

This report describes the experimental and analytical schemes, sample preparation, analytical methods, then summarizes and interprets the results.

2.0 EXPERIMENTAL SCHEME

To assess the effects on fish of potentially elevated environmental metals levels resulting from mining activity, a baseline emplacement study was proposed by George Derksen, project biologist for Conservation and Protection, Environment Canada, B.C. and Yukon Region. The original project called for an environmental emplacement study in the vicinity of a proposed mine site on Graham Island in the Queen Charlotte Islands, accompanied by an *in-situ* controlled mercury concentration exposure study, on subgroups of hatchery juvenile coho salmon. The controlled exposure experiment became infeasible, and the final study comprised a six-week emplacement of fish, obtained from the Pallant Creek Hatchery, at three stream sites: Middle Barbie Creek, Lower Barbie Creek and Gold Creek.

In addition to the emplaced fish, reference or control specimens of the hatchery population were taken at the inception of the emplacement study. Samples of the feral or wild coho populations were taken from each of the streams during the emplacement program. A subsidiary group of hatchery coho was obtained at the conclusion of the emplacement study to assess potential transport effects, because the initial control specimens were only obtained following transport from the hatchery.

The fish samples from the various groups, and water samples from each stream, as described below, were submitted to the CB Research International Corp. (CBR International) laboratory for the analyses discussed in Section 4.0. Some emplaced fish specimens were retained by Conservation and Protection for muscle tissue mercury analysis, and samples of supplemental feed, stream water and sediments were taken for comprehensive analysis at Conservation and Protection facilities.

2.1 EXPERIMENTAL CONDITIONS AND SAMPLE PARTICULARS

2.1.1 Day 0 Pallant Creek Hatchery Fish

Five composite samples of ten fish each were taken from the Pallant Creek Hatchery transport tank following a four hour transport, at ambient temperature, from the Pallant Creek Hatchery to Queen Charlotte City. Two additional composite samples of ten fish each, designated reference samples were obtained.

2.1.2 Emplacement Fish Study

At each of the study sites; Middle Barbie Creek (upstream of wetland), Lower Barbie Creek (lower wetland), and Gold Creek (downstream of Marie Lake), were located two steam-cleaned Aqua-Mesh 110 L Vexar-lined cages, suspended just below the surface, not touching the stream bed. Cages were loaded with thirty juvenile coho each, from the Pallant Creek Hatchery, averaging 12 g in weight. During the six week (42 day) exposure period, fish in each cage were supplemented with 15 g BioDiet (approximately 1.3 mm) pellet rations three times weekly. Stream velocity during the emplacement experiment varied from less than 2 cm/s to 10 cm/s. At the conclusion of the experiment, fish from each cage were divided into composite samples of ten fish each.

2.1.3 Feral Fish

Juvenile coho of sizes comparable to the emplacement of fish (10 to 12 g) were obtained at the emplacement sites during the *in-situ* caged study. On August 17, 1988, two, two and one composite samples (ten fish per composite) were taken from Middle Barbie, Lower Barbie and Gold Creeks, respectively. On September 9, 1988, five and two composite samples were taken from Lower Barbie and Gold Creeks, respectively.

2.1.4 Ancillary Transport Study: Day 42 Pallant Creek Hatchery Fish

At the conclusion of the emplacement experiment, additional hatchery fish were obtained. Five composite samples of ten fish each were taken directly from the hatchery raceways as a reference group. Transported fish were divided into two treatment groups of sixty fish each; one group at ambient temperature, wherein a $0.3^{o}C$ rise in temperature was recorded in the tanks over the five hour transport period; the other group at elevated temperatures, whereby hot water packs were placed under the tank liner, resulting in a $2.5^{o}C$ rise over the five hours. Each of the two groups was then divided into six composite samples of ten fish each.

2.1.5 Stream Water Samples

Water samples were taken at four locations, Lower Barbie Creek, Middle Barbie Creek, Florence Creek and Gold Creek, three times during the emplacement experiments, on August 6, August 23, and September 14, 1988.

2.2 SAMPLE PRESERVATION

2.2.1 Fish Specimens

All fish specimens were frozen in individual polyethylene bags immediately after sampling and maintained in a frozen state (-20^{o}C) until transport, or transported directly on dry ice to the CBR laboratory.

2.2.2 Water Samples

Water samples were taken in clean, amber glass containers and kept at approximately 5^{o}C during transport and following receipt at the CBR laboratory. Analysis for humic and/or fulvic substances was completed within 48 hours of receipt at the laboratory.

3.0 ANALYTICAL SCHEME FOR FISH TISSUE SAMPLES

3.1 RATIONALE

The use of metallothioneins as a biochemical indicator for metals has received much attention (Roesijadi, 1980; Vasak and Bauer, 1982; Roch *et al.*, 1982; Imber *et al.*, 1987). Metallothioneins are low molecular weight cysteine-rich proteins that have an affinity to bind several metals. These proteins can be induced by increased environmental concentrations of metals (Roch *et al.*, 1982). Other specific metal-binding ligands have also been studied. For example, Thomas and his collaborators (1983a,b) identified a low molecular weight cysteine-poor protein that binds cadmium in rainbow trout and is also induced by elevated metal levels.

In the environment, one must also consider the interaction of different metals and the effect they may have on an organism. In some bacteria for example, manganese transport proteins are partially blocked by increased cadmium concentrations (Perry and Silver, 1982). Manganese and cadmium, in this case, were competitive inhibitors of each other's transport.

A number of studies have focused on metal-metallothionein interactions as a monitoring tool for assessing metal toxicity *in situ* (Roch and McCarter, 1984; Imber *et al.*, 1987). Several authors have attempted to use increased metallothionein or protein induction and total metallothionein (MT) concentrations in marine and freshwater organisms to evaluate the exposure of an individual organism to elevated metal concentrations (Roch *et al.*, 1982; Roch and McCarter, 1984; Imber *et al.*, 1987). McCarter and his colleagues have shown that MT is a sensitive environmental indicator of metal stress. However, care should be used in the use of such systems for monitoring, as MT synthesis can also be induced by a number of non-metal stressors. Measurements of total MT without concomitant metal measurements could result in misleading conclusions about the degree of metal stress (Engel and Roesijadi, 1986).

Others have shown the importance of other ligand pools in assessing metal metabolism and toxicity (Mason and Nott, 1981; George, 1982; Simkiss and Mason, 1983; Sanders and Jenkins, 1984). Although most work has focused on cytosolic ligand pools, several non-cytosolic pools are also important in metal metabolism and toxicity. These non-cytosolic pools include the lysosomal vesicles and the membrane-bound granules (George, 1982; Simkiss and Mason, 1983). In contaminated localities, metals associated with the granular pool may

account for as much as 80 per cent of the total body burden of the organism (Mason and Simkiss, 1982). Existing data suggests that several indices derived from the above parameters may be particularly useful in determining metal stress.

From our own data taken from oyster (*Crassostrea gigas*) digestive tissue, we have developed (in consultation with Dr. Jenkins and his colleagues) the concept of molecular indices of metal stress (MIMS). In principle, simple determinations are made of metal bound to "membrane" or granular fraction, to metallothionein, and the low molecular weight pool. If the metabolism of an organism is stressed, the metal metabolism and therefore the ligand-bound distribution will change, causing a change in this ratio. For oysters, plots of "membrane-bound" copper versus total protein-bound copper, i.e., membrane + MT + low weight molecular protein (LWMP), produce a linear response. If metal stress, for example, causes a specific induction of metallothionein, this will be reflected in the data and will cause a change in the ratio.

In order to assess potential effects of elevated environmental metals levels on the basis of a MIMS approach, determination of metals associated with selected protein pools was required. In this particular instance, liver was the tissue of choice, in view of the larger concentrations of metals and metal-binding proteins/metallothionein present in relation to levels found in other tissues, gill or muscle, for example. Originally, the five metals of interest were: mercury, copper, zinc, cadmium and lead. The protein pools of interest were; low molecular weight (<20,000 Daltons) metal-binding proteins (metallothionein), intermediate weight cytosolic proteins (approximately 100,000 to 20,000 Daltons), and centrifugal pellet materials including granular structures, cellular debris and other heavy components. Total tissue metals determinations on homogenized liver tissue were to be conducted for confirmatory purposes.

In addition to the determination of metals and metallothionein concentrations, total protein and tissue dry weight determinations were carried out, to aid in normalization of the metals data.

Ideally, a matrix of the distribution of metals among the protein pools could be constructed for each test group. Differences between reference or control and emplacement or impacted site group data could indicate potential effects of mining activity in the test area.

3.2 ANALYTICAL SCHEME

The analytical scheme described in Figure 3.1 was proposed to determine the parameters required for the construction of a MIMS index.

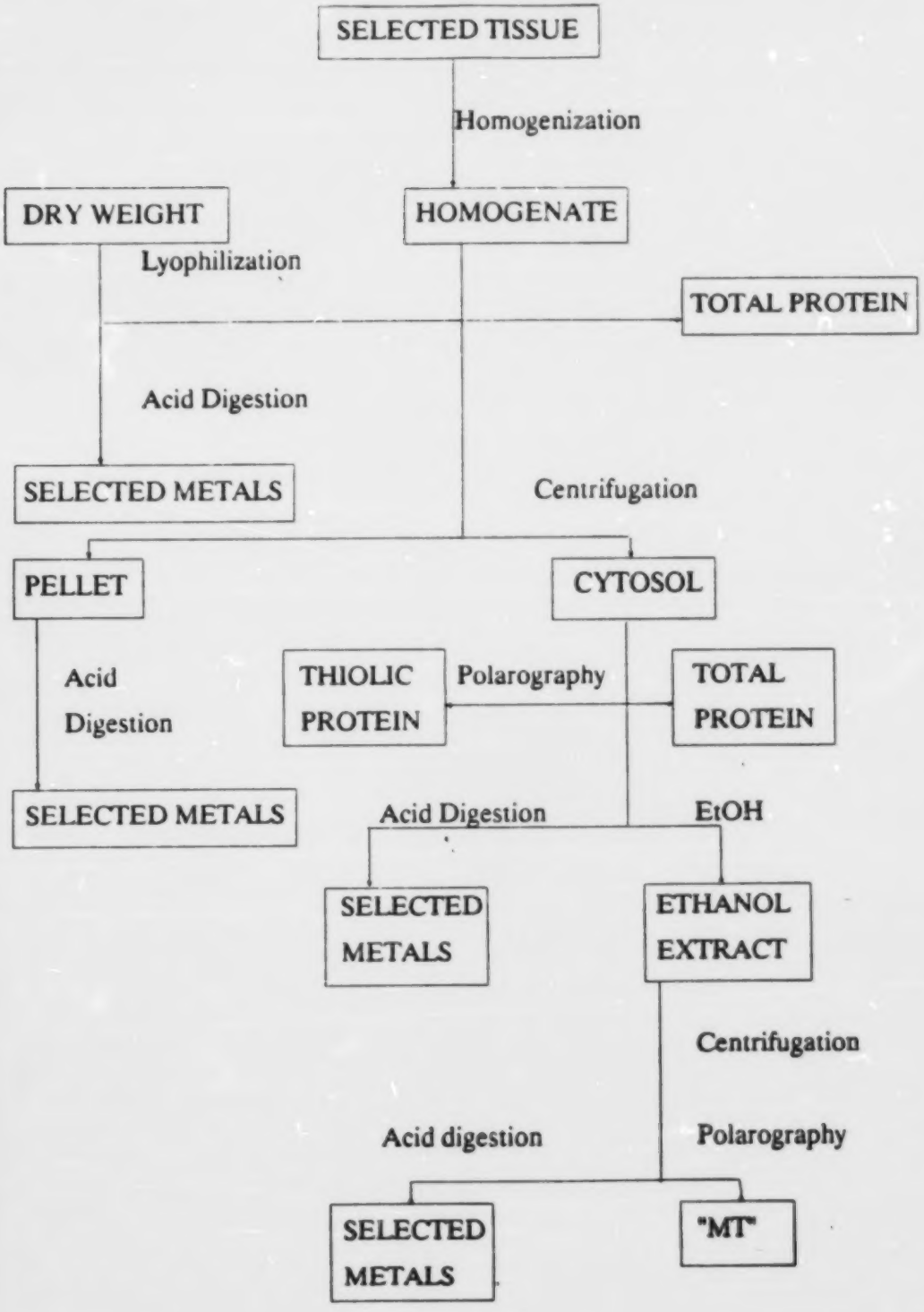

Figure 3.1 Analytical Scheme

A combination of factors, discussed further in Section 6.2, dictated changes in the number of analyses performed.

4.0 ANALYTICAL METHODS

4.1 REAGENT AND APPARATUS PREPARATION

4.1.1 Reagents

All chemicals used in preparation of reagents were ACS reagent grade or better. Acids used for sample digestions and subsequent analyses were ultra-pure (very low trace-metal content) Seastar Chemicals acids. Reagent grade water (Milli-Q) was in most cases prepared by reverse-osmosis pre-purification (Milli R/Q system, Millipore Corp.) followed by two-stage ion-exchange polishing (Milli-Q system, Millipore Corp.). Where trace metal analysis procedures required it, fresh distilled water was drawn from a sub-boiling quartz distillation unit using Milli-Q reagent grade water as feed.

4.1.2 Plastic and Glassware Preparation

All plastic and glassware used in sample preparation procedures, including sample aliquot containers, were acid-washed by soaking in 30% HNO_3 (Baker Instra-Analyzed grade, in Milli-Q) for at least eight hours followed by thorough rinsing with Milli-Q or quartz-distilled water.

Plastic and glassware used in sample preparation and analysis for mercury was prepared by soaking as above, in 30% HNO_3, with added $K_2Cr_2O_7$, followed by thorough rinsing with Milli-Q.

4.2 SAMPLE PREPARATION OF FISH SPECIMENS

4.2.1 Dissection

Fish were removed from storage (-20^{0}C or below) and placed, still in polyethylene bags, on beds of crushed ice. When partially thawed, individual fork lengths ($\pm$0.1 cm) and weights ($\pm$0.001 g) were recorded. Composite samples were selected at random from storage. Efforts were concentrated on completing dissections as quickly as possible and to maintain tissues at a low temperature (<5^{0}C). Dissections were carried out in individual polystyrene containers, using fresh

scalpel blades for each fish. Other dissecting tools (forceps, probes) were rinsed well with Milli-Q between each dissection. Fish livers were carefully excised to avoid puncture of adjacent gall bladder.

Individual liver weights (±0.0001 g) were recorded, and liver tissue was immediately immersed in known weights of chilled homogenization buffer (50 mM TRIS, pH 8.6, containing 2-mercaptoethanol as antioxidant, and 0.02% NaN_3 as microbial inhibitor) in acid-washed polyethylene test tubes. Any unusual observations as to the state of each liver were noted.

4.2.2 Homogenization

Composite samples of ten fish livers each (occasionally nine if a particular individual liver was not usable, or fifteen fish if the total liver tissue for ten fish was less than 0.6 g) were homogenized in a known weight of buffer. Additional buffer was added to create a ratio of roughly four parts of buffer per part of liver tissue. Samples were homogenized for one minute using a Brinkman Polytron Tissue Dismembrator at a setting of 4-5. During homogenization and all subsequent manipulations, samples were maintained at $<5^{o}C$ by storing on ice.

4.2.3 Sample Division

Following homogenization, samples were divided into the following portions:

a) a 2 g (± 0.0001 g) aliquot was transferred to a specially prepared 50 mL glass volumetric and immediately frozen, initially for dry weight determinations, followed by mercury analysis,

b) a 100 uL aliquot was retained in a 1.5 mL polyethylene centrifuge tube for total protein analysis,

c) a 1 g (± 0.0001 g) aliquot was transferred to an acid-washed polyethylene 1.5 mL centrifuge tube for centrifugation followed by further division and sample treatment,

d) any remaining homogenate was immediately frozen for metals analysis.

e) the aliquot described in c) was centrifuged at $5^{o}C$ for 30 minutes at 13,000 rpm (20,000 x g) in a Beckman Model J2-21 refrigerated centrifuge.

f) a portion of supernatant cytosol from centrifugation, 600 to 800 uL, was weighed (± 0.0001 g) into an acid-washed 10 mL screw-cap teflon reactor vial, and frozen for metal analysis.

g) another portion of supernatant cytosol approximately 400 ug (± 0.0001 g) was transferred to another acid-washed 1.5 mL centrifuge for denaturation with an equal weight of 95% ethanol.

h) a further 50 uL of supernatant cytosol was retained in separate 1.5 mL centrifuge tubes for total protein and thiolic protein analysis.

i) the pellet from centrifugation step e) was drained as well as practicable and frozen for metal analysis. Pellet weight (± 0.0001 g) was obtained by difference of initial dry tube weight and tube plus pellet weight.

j) following the denaturation step g), the mixture was allowed to stand for one hour at 5^{o}C, then centrifuged for fifteen minutes as described in e). A portion of the denatured cytosol ethanol extract, 700 ug, (± 0.0001g) was frozen in an acid-washed 1.5 mL centrifuge tube for metal analysis.

k) the remainder of ethanol extract was retained in another 1.5 mL centrifuge for metallothionein determination.

l) the centrifugation pellet from step j) was frozen.

Where possible, samples of adequate volume were divided to allow replicate determinations of various analyses (only one sample was large enough for replicate mercury analysis of homogenate portion).

4.3 QUALITY CONTROL

4.3.1 Buffer Blanks

During each day's processing of samples, portions of the homogenization buffer and reagent grade water were treated identically to composite liver samples, including homogenization and all subsequent sample division and treatment steps.

4.3.2 Standard Reference Material

A sample of DOLT-1 Dogfish Liver Standard Reference Material (NRC Marine Analytical Chemical Standards Program) was prepared to approximate the actual sample dilution of one part wet tissue to four parts of homogenization buffer. One g (± 0.0001 g) dry material was added to 24 g (± 0.0001 g) homogenization buffer. This stock mixture was allowed to hydrate for 0.5 hours at 5°C, then homogenized. Extra care was taken to agitate the stock each day to ensure representative sampling prior to withdrawing an aliquot for re-homogenization and further sample treatment. It is recognized that in most cases, sample aliquot volumes taken from this mixture were smaller than that recommended for 95% confidence level of analytical results.

4.4 HUMIC ACID ANALYSIS

The method used for the determination of humic/fulvic acid substance in stream waters as humic acid equivalents was based on that described by Carpenter and Smith (1984).

Samples (100 mL) were filtered through 0.2 um mixed ester membrane filters (MSI Corp.) in an all-glass filtration system (Nucleopore Corp.), and adjusted to pH 8 using 1 M NaOH. Sample absorbance at 365 nm, 1 cm glass cuvettes, versus reagent grade water was recorded using an LKB Ultraspec II UV/VIS spectrophotometer. A working curve used for the interpolation of sample readings was established using a range of concentrations of humic acid (Aldrich Chemicals) prepared in reagent grade water, adjusted to pH 8.

Iron concentrations of at least 2 mg/L may be tolerated in this spectrophotometric method without interfering with the determination of humic substances. Iron concentrations of filtered samples were semi-quantitatively determined by direct aspiration flame atomic absorption analysis, in comparison with a 2 mg/L iron solution (prepared from 1000 mg/L iron atomic absorption standard stock solution).

Limit of detection for the method is 0.01 mg/L humic acid.

4.5 PROTEIN DETERMINATIONS

4.5.1 Total Protein Analysis

The method used for the analysis of protein in total homogenate and cytosol portions of the fish liver tissue was that described by Bradford (1976).

Aliquots of sample (5 to 10 uL) or bovine serum albumin (BSA) stock solution (5 to 50 uL) were transferred to clean 10 mL glass culture tubes. Amounts of 50 mM TRIS homogenization buffer (50 uL) and Milli-Q were added to bring the total volume to 0.1 mL. Protein reagent (0.01% (w/v) Coomassie Brilliant Blue G-250, 4.7% (w/v) ethanol, 8.5% (w/v) phosphoric acid) was added (4.9 mL), and culture tube contents thoroughly mixed by vortexing.

Sample or standard absorbance at 595 nm was measured following a five to fifteen minute incubation time, in 1 cm glass cuvettes, versus a reagent blank of homogenization buffer and protein reagent, using an (LKB Ultraspec II UV/VLS) spectrophotometer. A standard curve was prepared using the range of BSA concentrations.

Fresh stock solution, 0.002 g BSA in 0.998 g homogenization buffer was prepared weekly and stored at $5^{o}C$.

4.5.2 Polarographic Methods for Thiolic Protein and Metallothionein

The method used was one originally developed by Brdicka (1933), and specifically applied to metal-binding proteins by Palechek and Pechan (1971), and improved by Thompson and Cosson (1984), based on the electrochemical reduction of the sulfur hydrogen bond present in thiolic proteins. Differential pulse polarographic analysis was carried out using a Metrohm (Brinkman) Polarograph VA 646 Processor and VA 647 Stand. Operating conditions are listed in Table 4.1.

Twenty milliliters of Brdicka support electrolyte (3.2 g $(NH_3)_6Co(III)Cl_3$ $3H_2O$ (Eastman) per litre of 1 M NH_4OH / 1 M NH_4Cl (BDH Assured grade) prepared in reagent grade water) with 75 uL of a 0.2% solution of Triton-X-100 surfactant (secondary maximum suppressant), was contained in a jacketed cell cooled to $6^{o}C$ ($\pm$ $0.5^{o}C$). A baseline scan was run prior to the addition of sample or standard. Analyses were performed on serial duplicate additions of appropriate volumes (2 to 10 uL) of either cytosol (thiolic protein) or denatured ethanol extract (comprised mostly of metallothionein) using fresh electrolyte for each sample. Printed curves of signal intensity (nA) as a function of applied potential were

Table 4.1 Metrohm 646 Operating Parameters for Thiolic Protein Analysis

PARAMETER	VALUE
Differential Pulse Mode	
Drop Size	5 units
Graphite Counter Electrode	
Ag/AgCl reference Electrode	3 M Salt Bridge
Drop Time	1 s
Initial Voltage	-1.200 V
Final Voltage	-1.752 V
Voltage Scan Step	6 mV
Pulse Amplitude	50 mV
Scan Rate	6 mV s^{-1}

obtained. Sample values were interpolated on a working curve prepared from measurements from a range of standard additions of a solution (50 mg/L) of Rabbit Metallothionein MT-II (Sigma Chemical Company) prepared in 10 mM Tris, pH 8.6, containing 2 mM 2-mercaptoethanol and 0.02% NaN_3. When not in use standard solution was stored at 5^{o}C.

4.6 MERCURY ANALYSIS

Total mercury concentrations were determined by a cold vapour atomic absorption technique following acid digestion (Environment Canada, 1979, Naquadat No. 80601). The data and interpretation of mercury analysis are presented in a compendium to the report.

4.7 METAL ANALYSIS

4.7.1 Sample Digestions

The following sample treatments were used on the four types of samples (total tissue homogenate, pellet material, cytosol and denatured ethanol extract) to produce digestates on which a number of elements could be determined.

4.7.1.1 Homogenates

Frozen total tissue homogenates retained in acid-washed test tubes were transferred to acid-washed 15 mL teflon reactor vials, using several rinses of 2N HNO_3 (Seastar), total volume 2 mL. Samples were digested on hotplates at 95^{o}C and taken to incipient dryness. Nitric (0.5 mL of 2N HNO_3) and perchloric (0.5 mL of concentrated $HClO_4$ (Seastar) acids were added and samples were again digested and sample volumes reduced to approximately 100 uL. Samples were then made to 5.0 mL with 1% HNO_3. Contents were well mixed prior to preliminary flame atomic absorption analysis and subsequent graphite furnace atomic absorption analysis.

4.7.1.2 Pellet Material

Pellet materials retained in microcentrifuge tubes were transferred to acid-washed 15 mL teflon reactor vials with several rinses of concentrated nitric acid (Seastar), total volume 2.0 mL. Samples were digested on a hotplate at 95^{o}C, until clear solutions were obtained. Concentrated perchloric acid (0.5 mL, Seastar) was

added, and samples were further digested and reduced to approximately 100 uL. Dilute nitric acid (1%) was used to make samples to a 5.0 mL final volume and digestates were well mixed prior to graphite furnace atomic absorption analysis.

4.7.1.3 Cytosol and Denatured Ethanol Extract

Samples were transferred from storage containers to acid-washed 10 mL polyethylene culture tubes using 2.5 mL 2N HNO_3 (Seastar). Samples were thoroughly mixed, allowed to stand overnight, then centrifuged for fifteen minutes at 4750 rpm using an IEC 428 Clinical centrifuge. Cytosol leachates were analyzed directly by flame atomic absorption methods. Denatured ethanol extract leachates were analyzed by graphite furnace atomic absorption techniques.

4.7.2 Flame Atomic Absorption Analysis

Sample digestates or leachates were analyzed by flame AA methods using a Varian AA-475 spectrophotometer. Usual instrumental parameters were employed. Sample copper concentrations were read directly, following input of calibration curve using a range of copper standards, prepared in appropriate matrices to match digestates or leachates, from concentrated copper (1000 mg/L) atomic absorption standard.

4.7.3 Graphite Furnace Atomic Absorption Analysis

Sample digestates or leachates were analyzed by furnace techniques using Varian GTA-95 with autosampler, in conjunction with a Varian AA-475 spectrophotometer. Partitioned pyrolytic coated graphite tubes without platforms were used in the furnace. Usual instrumental parameters were set up on Varian AA-475 (Table 4.2). The following program was used with the Varian GTA-95 to determine copper concentrations, using automatic calibration with a standard prepared in appropriate matrices.

Table 4.2 Furnace Operating Parameters

STEP NO.	TEMPERATURE ^{o}C	TIME SEC.	GAS FLOW	GAS TYPE
1	75	5.0	3.0	Normal
2	90	40	3.0	Normal
3	120	10	3.0	Normal
4	120	7.0	3.0	Normal
5	700	2.0	3.0	Normal
6	700	5.0	3.0	Normal
7	700	2.0	0.0	Normal
8	2300	1.1	0.0	Normal
9	2300	2.0	0.0	Normal
10	2300	1.0	3.0	Normal

5.0 RESULTS

Methods for the analyses reported in this section are contained in Section 4.0.

5.1 STREAM WATER SAMPLES

The water samples collected by EP at the four sites were analyzed for humic and fulvic acid substances as humic acid equivalents using humic acid (Aldrich Chemicals) as the reference material. Concentrations of humic/fulvic substances were such that pre-concentration was not required. [Corrections for interfering iron species was not required as sample iron concentrations were less than 2 mg/L]. Table 5.1 displays the data.

5.2 PHYSIOLOGICAL PARAMETERS

Individual weights (± 0.001 g), fork lengths (± 0.1 cm) and fish liver weights (± 0.0001 g) were recorded for each specimen supplied by CP. The data are contained in Appendix A.

5.3 PROTEIN ANALYSIS

Analysis of total protein in both homogenate and cytosol portions and polarographic determination of thiolic protein and metallothionein was conducted as soon as possible following sample preparation and division, normally within 24 hours. Tables 5.2A through 5.2D show the data for the control, transport study, emplacement and feral specimen sample groups, respectively.

5.4 METAL ANALYSES

Determination of copper was conducted on total tissue (homogenate), pellet, cytosol and ethanol extract (metallothionein) material from four selected groups of composite samples. Tables 5.3A and 5.3B list the data for the reference material DOLT-1, and the selected sample groups, respectively.

Table 5.1 Stream Sample Humic Substance Content (Aldrich equivalent mg/L)

SAMPLE SITE CREEK	DATE AUGUST 6	AUGUST 23	SEPTEMBER 16
LOWER BARBIE	48	66	43
MIDDLE BARBIE	35	61	45
FLORENCE	32	55	37
GOLD	6	5	5

Table 5.2A Protein Analyses: Day 0 Pallant Creek Hatchery Control Fish

SAMPLE GROUP		TOTAL PROTEIN (all values ug/mg wet liver tissue) HOMOGENATE	TOTAL CYTOSOL	POLAROGRAPHIC PROTEIN THIOLIC	METALLO-THIONEIN
CONTROL	1	111.0 (1.7)[a]	103.5 (5.2)	1.016 (.023)	.373
	2	106.2	110.5	.952	.274
	3	115.8 (10.6)	121.4		.325
	4	115.7 (6.2)	119.3	1.315 (.674)	.234
	5	105.6 (1.7)	133.4	.784	.241
	6	98.5	110.4	1.217 (.576)	.198
	7	99.3 (.0)	127.2	1.320 (.569)	.249 (.013)
OVERALL	x	107.5	118.0	1.101	.271
	s	6.6	9.7	.199	.055

NOTE:
a) Parenthesized values indicate standard deviation of triplicate analyses.

Table 5.2B Protein Analyses: Day 42 Pallant Creek Hatchery Transport Study Fish

SAMPLE GROUP		TOTAL PROTEIN (all values ug/mg wet liver tissue) HOMOGENATE	TOTAL CYTOSOL	POLAROGRAPHIC PROTEIN THIOLIC	METALLO-THIONEIN
NO TRANSPORT	1	87.7	108.3	1.271	.332
	2	85.0	105.0	1.25	.309
	3	82.3	75.7	1.052	.246
		(5.8)[a]	(12.5)	(.037)	(.017)
	4	87.4	94.3	1.542	.243
	5	81.6	89.6	1.308	.177
	x	84.8	94.6	1.285	.261
	s	2.5	11.7	.156	.055
TRANSPORT (AMBIENT)	6	101.6	83.7	1.268	.366
		(4.0)	(4.4)		
	7	83.1	79.7	1.562	.243
	8	80.2	97.4	1.424	.388
	9	86.5	77.4	1.340	.249
	10	83.8	71.1	1.271	.263
	x	87.0	81.9	1.373	.302
	s	7.5	8.8	.110	.062

Continued on next page.

Table 5.2B Continued. Protein Analyses: Day 42 Pallant Creek Hatchery Transport Study Fish

SAMPLE GROUP		TOTAL PROTEIN (all values ug/mg wet liver tissue) HOMOGENATE	TOTAL CYTOSOL	POLAROGRAPHIC PROTEIN THIOLIC	METALLO-THIONEIN
TRANSPORT (ELEVATED T)	11	55.5 [1.9][b]	69.8 [3.3]	1.388	.307
	12	74.5 [6.2]	81.9 [6.5]	1.434	.378
	13	58.8 [6.6]	80.1 [1.7]	1.212	.295
	14	89.8 [6.6]	80.5 [1.7]	1.276 (.063)	.234
	15	77.6 [1.1]	58.5 [0.3]	1.336	.280
	x	71.2	74.2	1.329	.299
	s	12.6	9.0	.079	.047
OVERALL	x	81.0	83.5	1.329	.287
	s	11.1	13.0	0.125	.058

NOTES:
a) Parenthesized values indicate standard deviation of triplicate analyses.
b) Square bracketed values indicate range of duplicate determinations.

Table 5.2C Protein Analyses: Day 42 Emplacement Fish

SAMPLE GROUP		TOTAL PROTEIN (all values ug/mg wet liver tissue) HOMOGENATE	TOTAL CYTOSOL	POLAROGRAPHIC PROTEIN THIOLIC	METALLO-THIONEIN
MIDDLE BARBIE CREEK	16	77.2	74.8	.978	.295
	17	71.0 (3.2)[a]	66.8 (3.7)	.932	.257
	18	91.9	77.8	1.244	.274
	19	69.9	66.7	.973 (.063)	.223
	20	83.4 [0.1][b]	70.6 [5.8]	.998	.266
	x	78.7	71.3	1.025	.263
	s	8.2	4.4	.111	.023
LOWER BARBIE CREEK	22	81.5	79.3	1.005	.277
	23	79.3 (1.7)	76.0 (4.8)	1.186	.323
	24	52.8	77.2	1.073	.233
	25	83.7	78.8	1.232	.282
	26	84.9 [8.6]	77.1 [2.0]	1.196	.271
	x	76.4	77.7	1.138	.277
	s	12.0	1.2	.085	.029

Continued on next page.

Table 5.2C Continued. Protein Analyses: Day 42 Emplacement Fish

SAMPLE GROUP		TOTAL PROTEIN (all values ug/mg wet liver tissue)		POLAROGRAPHIC PROTEIN	
		HOMOGENATE	TOTAL CYTOSOL	THIOLIC	METALLO-THIONEIN
GOLD CREEK	28	72.9	67.2	1.065	.260
	29	69.2	67.2	1.024	.170
	30	84.0	68.9	1.035	.241
	31	66.5	78.8	1.078	.151
	32	78.5	77.1	1.101	.247
		(4.3)	(7.7)		
	x	74.2	71.9	1.061	.214
	s	6.3	5.0	.028	.044
OVERALL	x	76.4	73.6	1.075	.251
	s	9.3	4.9	.095	.043

NOTES:

a) Parenthesized values indicate standard deviation of triplicate analyses.

b) Square bracketed values indicate range of duplicate determinations.

Table 5.2D Protein Analyses: Feral Fish

SAMPLE GROUP		TOTAL PROTEIN (all values ug/mg wet liver tissue) HOMOGENATE	TOTAL CYTOSOL	POLAROGRAPHIC PROTEIN THIOLIC	METALLO-THIONEIN
LOWER BARBIE CREEK AUG 17/88	43	76.2 (4.4)[a]	84.8 (12.6)	1.027	.190
	44	80.8	93.4	.936	.177
	x	78.5	89.1	.982	.183
	s	2.3	4.3	.046	.007
MIDDLE BARBIE CREEK Aug 17/88	45	71.7 [2.4][b]	80.4 [1.2]	.975	.150
	46	66.4	87.1	.923	.155
	x	69.0	83.8	.949	.152
	s	2.6	3.4	.026	.002
LOWER BARBIE CREEK SEPT 9/88	34	56.5	50.0	.604	.091
	35	90.4	75.8	.999	.172
	36	70.2 [0.7]	70.0 [3.4]	1.009	.167
	37	77.6 [1.5]	62.0 [0.8]	.971	.201
	38	83.1	83.8	1.030	.191
	x	75.6	68.3	.923	.164
	s	11.6	11.6	.160	.039

Continued on next page.

Table 5.2D Continued. Protein Analyses: Feral Fish

SAMPLE GROUP		TOTAL PROTEIN (all values ug/mg wet liver tissue) HOMOGENATE	TOTAL CYTOSOL	POLAROGRAPHIC PROTEIN THIOLIC	METALLO-THIONEIN
GOLD CREEK	40	77.1	70.3	1.106	.205
AUG 1988	41	92.3	102.6	1.119	.160
SEP 9/88	48	77.4 [0.4]	82.2 [5.0]	1.287	.257
	x	82.3	85.0	1.171	.207
	s	7.1	13.3	.082	.040
OVERALL	x	76.6	78.5	.999	.176
	s	9.5	14.1	.152	.038

NOTES:

a) Parenthesized values indicate standard deviation of triplicate analyses.

b) Square bracketed values indicate range of duplicate determinations.

Table 5.3A Copper Concentrations of Buffer Blanks and Reference Material DOLT-1

BUFFER BLANK (Digestate Cu(ng/g))

TOTAL TISSUE	PELLET	CYTOSOL	DENATURED EXTRACT
<3	<3	<3	<3
<3	<3	<3	<3
<3	<3	<3	<3
<3	<3	<3	<3

REFERENCE MATERIAL DOLT-1 (Copper Content (20.8 $\pm$ 1.2 ug/g))

	TOTAL TISSUE Cu (ug/g)	PELLET Cu (ug/g)
	15.6	1.2
	15.8	1.7
	17.1	2.5
	17.6	
	19.6	
x	17.1	
s	1.4	

Cu recovery = 82.2%

Table 5.3B Copper Concentrations in Protein Pools (in Terms of Wet Tissue Concentrations).

SAMPLE GROUP	TOTAL TISSUE	PELLET (P) (ug/g)	CYTOSOL (C) (ug/g)	P/C (ug/g)	DENATURED EXTRACT (DE) (ug/g)	DE/C
DAY 0 PALLANT CREEK - CONTROL						
1	8.14	2.03	8.30	0.24	11.71	1.41
2	9.59	2.13	10.59	0.20	12.46	1.18
3	7.90	1.73	10.15	0.17	9.95	0.98
4	7.47	1.54	8.67	0.18	12.15	1.40
5	11.88	2.22	14.08	0.16	15.84	1.13
x	9.00	1.93	10.36	0.19	12.42	1.22
s	1.80	0.29	2.29	0.03	2.14	0.18
DAY 42 EMPLACED - LOWER BARBIE CREEK						
22	8.58	1.90	10.13	0.19	10.14	1.00
23	9.37	2.53	12.02	0.21	12.29	1.02
24	7.53	1.96	10.10	0.19	8.71	0.86
25	10.38	2.39	11.35	0.21	14.54	1.28
26	9.35	2.26	9.25	0.24	10.72	1.16
x	9.04	2.21	10.57	0.21	11.28	1.06
s	1.06	0.27	1.10	0.02	2.23	0.16
DAY 42 EMPLACED - GOLD CREEK						
28	9.18	2.26	12.02	0.19	12.49	1.04
29	7.89	2.07	10.97	0.19	9.83	0.90
30	8.79	2.24	11.37	0.20	11.89	1.05
31	9.36	2.63	13.09	0.20	15.86	1.21
32	10.59	2.69	13.16	0.20	16.84	1.28
x	9.16	2.38	12.12	0.20	13.38	1.10
s	0.98	0.27	0.99	0.01	2.90	0.15
FERAL FISH - LOWER BARBIE CREEK (Sept 1988)						
34	2.07	1.13	2.66	0.42	2.15	0.81
35	--	1.92	5.87	0.33	3.82	0.65
36	2.83	1.33	5.82	0.23	3.79	0.65
37	3.19	1.41	4.17	0.34	4.31	1.03
38	3.62	0.96	2.79	0.34	2.53	0.91
x	2.93	1.35	4.26	0.33	3.32	0.81
s	0.66	0.36	1.56	0.07	0.93	0.17

Table 5.3C represents a copper mass budget for the pellet and cytosol protein pools. The total mass is the sum of the masses of pellet and cytosol components:

M total = M pellet + M cytosol,

where

M pellet (ug) = mass of pellet component (g) x pellet Cu conc. (ug/g)

and

M cytosol (ug) = mass of cytosol component (g) x pellet Cu conc. (ug/g)

Table 5.3C Mass Budget of Copper (ug Cu present in liver tissue component)

SAMPLE GROUP	PELLET Cu	CYTOSOL Cu	TOTAL Cu	P/C
DAY 0 PALLANT CREEK - REFERENCE				
1	.070	1.687	1.757	0.041
2	.085	2.313	2.398	0.037
3	.075	2.198	2.273	0.034
4	.065	1.819	1.884	0.036
5	.105	2.863	2.968	0.037
x	.080	2.176	2.256	0.037
s	.014	.417	.428	0.003
DAY 42 EMPLACED - LOWER BARBIE CREEK				
22	.130	1.878	2.008	0.069
23	.080	2.771	2.851	0.029
24	.120	1.963	2.083	0.061
25	.125	2.172	2.297	0.058
26	.090	2.054	2.144	0.044
x	.109	2.168	2.277	0.052
s	.020	.317	.302	0.016
DAY 42 EMPLACED - GOLD CREEK				
28	.105	2.413	2.518	0.044
29	.110	2.032	2.142	0.054
30	.155	2.265	2.420	0.068
31	.185	2.297	2.482	0.081
32	.130	2.580	2.710	0.050
x	.137	2.317	2.454	0.059
s	.030	.180	.184	0.015
FERAL FISH - LOWER BARBIE CREEK				
34	.055	.971	1.026	0.057
35	.080	1.220	1.300	0.066
36	.060	1.216	1.276	0.049
37	.085	.758	.843	0.112
38	.045	.636	.681	0.071
x	.065	.960	1.025	0.071
s	.015	.236	.241	0.024

6.0 INTERPRETATION

6.1 SAMPLE PREPARATION AND DIVISION

Based on previous analyses of archived fish liver homogenates, the estimated sample size (0.6 g composite) weight was believed to be sufficient for all analyses. This, however, proved not to be the case. There was adequate sample for all required protein analysis and the four metals (Cu, Cd, Zn, Pb) associated with various protein pools, but insufficient sample to conduct mercury determinations on all protein pools. Even if all sample homogenate for other analyses was re-allocated for mercury analysis, mercury analysis on all protein pools would not have been possible. An alternative approach, pooling composite samples within sample groups to provide adequate sample volumes was not considered viable, as this would drastically reduce the number of data available for statistical comparison of groups. Based on the knowledge that although mercury was the primary element of interest, the other metal distribution data and the MIMS approach to analysis was such a vital aspect of the original contract, it was concluded that protein and other metals data were too important to sacrifice. In addition, it was known (personal communication with Scientific Authority) that CP was conducting total mercury analysis on muscle tissue of retained emplacement specimens. The decision was made to divide the sample, as described in Section 4.2.3, to allow determination of four metals among protein pools, and total mercury determination on total liver tissue homogenate only. The mercury results are presented in a compendium to this report.

6.2 PROTEIN ANALYSIS

6.2.1 Metallothionein Trends

Analytical requirements dictated that determination of protein components, total thiolic and metallothionein, be conducted as soon as possible following sample preparation to avoid any possible denaturation or degradation of such. Thus, protein data was quickly produced. Inspection of this data provided an extremely interesting (and unforeseen) observation: metallothionein levels were lowest in the feral coho population, roughly half the level of the hatchery fish. Tables 6.1 and 6.2 summarize the data and t-test statistical differences respectively.

Table 6.1 Summary of Metallothionein Data

SAMPLE GROUP	METALLOTHIONEIN (ug/g wet tissue)	STANDARD DEVIATION (n)
DAY 0 PALLANT CREEK FISH		
Reference	0.271	0.055 (7)
Day 42 Transport Study Day 42 Fish		
Reference	0.261	0.055 (5)
Cooled	0.302	0.062 (5)
Ambient	0.299	0.047 (5)
Overall	0.287	0.058 (15)
DAY 42 EMPLACEMENT FISH		
Middle Barbie Creek	0.263	0.023 (5)
Lower Barbie Creek	0.277	0.029 (5)
Gold Creek	0.214	0.044 (5)
Overall	0.251	0.043 (15)
FERAL FISH		
Middle Barbie Creek	0.152	0.002 (2)
Lower Barbie Creek	0.170	0.034 (7)
Gold Creek	0.207	0.040 (3)
Overall	0.176	0.038 (12)

Table 6.2 Summary of T-Test Results for Statistical Differences in Metallothionein Levels (one tail test)

			p=0.05
Day 0 Control	<	Day 42 PC No Transport	No
Day 0 Control	<	Day 42 PC Ambient Transport	No
Day 0 Control	<	Day 42 PC Elevated Transport	No
Overall Emplaced	<	Day 0 Control	No
Feral	<	Overall Emplaced	Yes
Feral	<	Day 0 Control	Yes
Gold Creek Emplaced	<	Mid Barbie Emplaced	Yes
Gold Creek Emplaced	<	Lower Barbie Emplaced	Yes
Gold Creek Emplaced	<	Day 0 Control	No
Gold Creek Feral	<	Gold Creek Emplaced	No

It is our experience (supported by numerous workers in this field of research) that the trend most commonly seen is that metal-binding protein levels (regardless of organism, exact protein characterization or specific metals bound) generally are lowest in pristine or in rigidly controlled low trace metal concentration situations, and higher in impacted or elevated metal concentration environments. When biological specimens, normally of a known history or "clean" origin and of known metal-binding protein levels, are emplaced in an environment of elevated metal concentrations, metal-binding protein production is induced (Roesijadi, 1980). This can be observed by monitoring thiolic proteins, most specifically metallothionein, polarographically without requiring specific determination of metals associated with the protein.

This general or first-tier monitoring approach can be used with success in a (tentative) classification of impacted or non-impacted sites, provided the requisite background metal-binding protein levels are known. A higher level approach, such as MIMS analysis, involves a more detailed description of the system, e.g., distribution of various metals among protein pools.

In this particular case, control (Day 0) and overall emplaced specimens have significantly higher metallothionein levels than wild populations, thus implying that greater metal stressor levels existed in the hatchery environment. There was, however, no noticeable decrease in metallothionein levels between Day 0 control and Day 42 emplaced specimens. A lengthier emplacement schedule might conceivably have yielded more information on possible degradation rates of metallothionein when changing from an apparent higher (hatchery) to lower (wild) stressor environment.

Although Gold Creek emplaced specimens had significantly lower metallothionein levels than those of Middle and Lower Barbie Creek emplaced fish, they were statistically similar to Day 0 control and Gold Creek feral populations. This suggests potential environmental stressor differences between water systems on the induction and maintenance of metallothionein, and/or perhaps even a natural variability of hepatic metallothionein levels between fish populations assumed to come from unstressed environments. Consequently, the need arises for baseline studies of fish populations in each water system that might be subjected to metal pollution at a future time. In addition, larger sample sizes would ensure more accurate statistical assessment of difference variability between groups.

As all sites were presumably of clean origin, the differences in metallothionein levels discussed above would indicate that the next logical step would be the determination of the metal distribution among protein pools.

6.2.2 Total Protein Analysis and Data Normalization

Total protein analysis in homogenate and cytosol proteins was conducted. These concentration data were to be used in an effort to normalize the data and thus reduce the standard deviations of other data groups caused by high individual variability commonly observed in biological populations.

It was attempted to normalize the wet tissue metallothionein values, which had group relative standard deviations of roughly 20%. Normalizations relating to thiolic protein alone, and in combination with average total protein were conducted.

The results of the described normalizations are shown in Appendix 2. The attempted normalizations do not reduce the standard deviation of the metallothionein data.

6.3 METAL ANALYSIS

Copper analyses were performed on various protein pools to provide a MIMS type baseline overview. Concentration data is presented in Section 5.5 and the copper mass budget for pellet and cytosol protein pools is summarized in Table 6.3. Relevant statistical t-test results are found in Table 6.4.

Most of the copper appears to be bound in the cytosol pool, that fraction comprised of soluble materials < 100,000 Daltons, with the pellet material, comprised of heavy proteins > 100,000 Daltons, granular structures and other cellular debris, accounting for the remainder of the copper. A higher percentage of cytosol copper was observed in the control fish (96.5%) than in the emplaced (94.4% to 95.2%) or feral fish (93.7%).

The denatured pellet was not analyzed for copper and hence a mass copper budget for the cytosol ligand pool (M cytosol = M denatured pellet + M denatured cytosol) could not be accurately assessed. Nonetheless, from the distribution of copper concentrations of the denatured and cytosol components given in Table 5.3B, it would appear that in the control and emplaced fish the denaturation process, which precipitates out most proteins > 20,000 Daltons, does not remove as great a proportion of the copper associated with the cytosol as noted in the feral group. Thus, the copper would appear to be primarily associated with metallothionein and very low molecular weight ligands in these two groups of fish. Conversely, in the feral fish group, the lower DE/C ratio (denatured extract/cytosol) implies a greater concentration of copper in the pellet material

Table 6.3 Group Average Copper Mass Budget and Percentage Distribution of Protein Pools

SAMPLE GROUP	PELLET (ug)	CYTOSOL (ug)	TOTAL (ug)	P/C	PERCENT PELLET	PERCENT CYTOSOL
REFERENCE DAY 0 PALLANT CREEK	0.80 (.014)[a]	2.176 (.414)	2.256 (.428)	0.037 (0.003)	3.5	96.5
EMPLACEMENT DAY 42 LOWER BARBIE	.109 (.020)	2.168 (.317)	2.277 (.302)	0.052 (0.016)	4.8	95.2
EMPLACEMENT DAY 42 GOLD CREEK	.137 (.030)	2.317 (.180)	2.454 (.184)	0.059 (0.015)	5.6	94.4
FERAL LOWER BARBIE	.065 (.015)	.960 (.236)	1.025 (.241)	0.071 (0.024)	6.3	93.7

NOTES: a) Parenthesized values represent standard deviation (s), n=5.

Table 6.4 Summary of T-Test Results for Statistical Differences of Copper Levels in Various Protein Pools (one tail test)

			p=0.05
Copper Concentration DE/C			
Emplaced	<	Control	No
Feral	<	Overall Emplaced	Yes
Feral	<	Day 0 Control	Yes
Mass Budget Copper P/C			
Day 0 control	<	Overall Emplaced	Yes
Day 0 control	<	Feral	Yes
Overall emplaced	<	Feral	No

precipitated by the denaturation process. This suggests that there is more copper associated with moderate weight (between 100,000 and 20,000 Daltons) ligands in the feral fish in comparison with the amount of copper associated with these ligands in the hatchery fish specimens.

A comparison of the overall copper levels reveals a considerable difference between feral fish and the control or emplaced fish. The copper concentration (wet tissue) in feral fish liver tissue is only one-third of that of the other two groups; mass copper results are one-half of the emplaced and control groups. These comparisons parallel the results of the metallothionein determinations, wherein feral fish have significantly lower levels of this protein pool than do the emplaced and control groups. The inference could be made that the higher levels of metallothionein existing in the hatchery fish could be due to increased copper loading, and that this metal may be more important than mercury as a source of metallothionein induction.

Notable is the trend towards a higher pellet/cytosol (P/C) mass ratio in the emplaced fish, approaching that of the feral fish group. The metallothionein copper (denatured extract)/cytosol copper (DE/C) concentration ratios show a decreasing trend from control and emplacement to feral fish groups. These two observations suggest that copper is being re-distributed from the low molecular weight metallothionein pool to the moderate (100,000 to 20,000 Dalton) molecular weight pool.

Investigations of this copper distribution over a longer time frame and in various other tissues should be made to affirm these trends in redistribution of copper in the emplaced fish and to study the transport mechanism between tissues. These mechanisms may allow for the eventual excretion of accumulated metal in a less stressed environment.

6.4 EFFECTS OF TRANSPORTATION ON METALLOTHIONEIN LEVELS

Some concern was expressed by the Scientific Authority that transportation at ambient temperature of initial reference specimens could have resulted in the fish being exposed to undue stress and possible changes in metallothionein levels. However, a statistical comparison by t-test of Day 42 hatchery specimens indicated no significant differences ($p=0.05$) in the metallothionein observed between non-transported, ambient T transported and elevated T transported samples. As well, metallothionein levels of Day 42 fell within the range of variability

of Day 0 levels. The effect of transportation would be a short term phenomenon with respect to the longer time frame of the emplacement experiment. It is more likely that any effects of increases in ambient temperature would show up in a heat-shock stress protein study.

7.0 CONCLUSIONS

This report presents the results of a baseline emplacement study conducted in the vicinity of a proposed mining site on Graham Island in the Queen Charlotte Islands. The development of a data base to assess effects on fish of potentially elevated environmental metals, specifically mercury, from mining action were to be determined using the MIMS approach of metal speciation of various protein pools.

A first-tier approach, studying the metallothionein levels of three groups of fish (control, emplaced, and feral), revealed a significant decrease in actual metallothionein levels between hatchery and feral fish. Contrasted to this, tentative results suggested mercury levels of total hepatic tissue were elevated in feral populations, and in the muscle tissue of emplaced versus control fish, as analyzed by CP (refer to compendium).

Metal speciation of copper had been performed on the total protein, pellet (> 100,000 Daltons) cytosol (< 100,000 Daltons) and metallothionein or denatured (< 20,000 Daltons) protein pools. Differential binding of copper in these pools occurred with the various fish groups. Feral fish had the least amount of mass copper in all pools, the lowest denatured/cytosol ratio, and the highest pellet/cytosol ratio. Emplaced fish showed a trend towards a higher pellet/cytosol ratio.

From the observations presented here, metal speciated protein pools and metallothionein trends do not appear to indicate substantial environmental stress. It was not possible to show changes in metallothionein levels with parallel re-distribution of copper in emplaced fish. A longer time frame for the emplacement experiment, larger sample size and considerations of age, condition factors, food source, other metal stressors (Cd, Zn, Pb), genetic variability between populations, and environmental stressor variability between creek systems are among the factors that could contribute to a better understanding of the complex processes of induction and degradation of metallothionein and of transport mechanisms that result in accumulation and re-distribution of metals in protein pools.

8.0 RECOMMENDATIONS

Because of budget constraints and very small sample tissue sizes it was not possible to provide a complete MIMS study with mercury and the four metals, copper, zinc, cadmium and lead, nor to provide dry weight determinations. With additional funding, however metals analyses of zinc, cadmium and lead could be performed on the remaining metal digestate.

Recommendations and observations for future studies are as follows:

1. Mercury. At least 3 g liver tissue is required to determine mercury levels in all protein pools. The analytical method will also have to be modified to provide greater accuracy and precision of the data.

2. Metals. While 0.6 g tissue is sufficient for analysis of the four metals, copper, zinc, cadmium and lead, more sample would be required for additional metal analyses.

3. Dry Weights. Dry weight determinations would provide a better normalization parameter for metallothionein than the total protein analyses by spectrophotometry.

4. Lipid Analyses. Results from a previous study have suggested that an inverse correlation may exist between lipid loading and metallothionein levels. Hence, lipid determinations, providing a measure of condition factor, may also be used as a normalization parameter.

5. Organic Mercury. Exposure, or induction, and MIMS studies have mostly focussed on inorganic metal stressors. A lack of such information exists for organic-metallic stressors, in relation to initial response to exposure and subsequent transport or detoxification mechanisms. Thus, a future study could potentially incorporate a comparison of inorganic mercury to organo-mercury as stressors.

6. Copper Complexing Capacity. Copper complexing capacity (CCC) measurements of stream humic substances provides an excellent estimate of metal ion complexing capacity. This is critical in understanding metal ion transport and bioavailability. CCC values are readily obtained by titration of stream water samples with copper ions at pH 5. Titration progress is followed by anodic stripping voltammetry (ASV).

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APPENDIX 1

Physiological Parameters

Table A1.1 Day 0 Pallant Creek Hatchery Fish

SAMPLE	FORK (cm)	FISH WEIGHT (g)	LIVER (g)
PCR1 -A	9.2	15.605	.2829
-B	8.9	13.466	.2401
-C	8.2	10.065	.0992
-D	8.2	10.762	.1292
-E	9.4	14.131	.2002
-F	8.7	11.842	.1852
-G	8.9	11.522	.1248
-H	8.4	10.133	.1391
-I	8.0	10.034	.0895
-J	8.4	9.789	.1063
x	8.6	11.735	.1597
s	.4	1.917	.0616
PCR2 -A	9.2	10.396	.1191
-B	9.1	13.060	.2146
-C	8.6	10.244	.2443
-D	8.2	9.333	.2092
-E	9.2	12.892	.2882
-F	8.1	8.811	.1352
-G	8.6	9.664	.1183
-H	9.2	15.837	.2765
-I	9.2	13.171	.2180
-J	10.5	15.674	.2803
x	9.0	11.908	.2104
s	.6	2.446	.0625
PCR3 -A	9.3	11.405	.2241
-B	9.8	16.392	.2670
-C	9.2	13.624	.2267
-D	9.2	13.453	.2561
-E	9.2	12.577	.1879
-F	9.3	15.255	.2104
-G	8.3	8.658	.1156
-H	9.3	12.397	.1735
-I	9.1	11.573	.1836
-J	9.0	11.703	.2189
x	9.2	12.704	.2064
s	.4	2.049	.0415

Continued on next page.

Table A1.1 Continued. Day 0 Pallant Creek Hatchery Fish

SAMPLE	FORK (cm)	FISH WEIGHT (g)	LIVER (g)
PCR4 -A	9.3	10.788	.0645
-B	10.5	17.398	.1689
-C	9.3	10.908	.0997
-D	10.4	15.461	.2600
-E	9.7	13.191	.1295
-F	9.5	12.557	.1484
-G	9.4	11.642	.1366
-H	9.1	9.781	.1173
-I	8.6	9.249	.0805
-J	8.2	7.738	.1067
x	9.4	11.871	.1312
s	.7	2.759	.0521
PCR5 -A	8.3	11.513	.1658
-B	7.9	7.456	.0735
-C	7.9	8.234	.0964
-D	8.6	11.222	.2256
-E	8.2	8.794	.1051
-F	8.2	8.705	.1193
-G	9.7	16.500	.2287
-H	10.1	17.319	.2872
-I	8.0	7.874	.0574
-J	7.1	7.454	.0708
x	8.4	10.507	.1430
s	.8	3.475	.0755
PCR6 -A	8.9	10.438	.1490
-B	8.5	8.607	.0858
-C	8.3	9.662	.1519
-D	8.9	9.750	.1299
-E	9.3	12.733	.1024
-F	9.0	9.813	.1078
-G	9.5	11.475	.0669
-H	8.2	7.885	.0688
-I	8.3	8.231	.0940
-J	7.4	6.547	
x	8.6	9.514	.1063
s	.6	1.707	.0298

Continued on next page.

Table A1.1 Continued Day 0 Pallant Creek Hatchery Fish

SAMPLE	FORK (cm)	FISH WEIGHT (g)	LIVER (g)
PCR7 -A	9.8	13.957	.1404
-B	9.2	9.715	.1153
-C	10.1	15.288	.0570
-D	8.9	9.958	.1141
-E	9.3	11.233	.1269
-F	9.3	11.710	.0865
-G	9.3	10.712	.0756
-H	8.3	7.915	.0620
-I	8.8	11.067	.2267
-J	9.2	12.069	.0563
x	9.2	11.362	.1061
s	.5	1.997	.0494

Table A1.2 Day 42 Pallant Creek Hatchery Fish

SAMPLE		FORK (cm)	FISH WEIGHT (g)	LIVER (g)
1	-A	10.4	15.309	.1517
	-B	10.2	15.386	.1680
	-C	10.6	17.356	.1320
	-D	11.6	22.494	.3442
	-E	8.9	9.922	.0866
	-F	10.6	16.474	.1521
	-G	10.4	16.205	.1714
	-H	9.9	12.058	.1124
	-I	10.4	20.485	.3022
	-J	10.5	15.942	.1737
	x	10.4	16.163	.1794
	s	.6	3.427	.0770
2	-A	11.2	20.443	.2817
	-B	11.6	23.383	.2574
	-C	11.5	19.928	.1785
	-D	10.6	15.817	.1551
	-E	10.9	17.043	.1143
	-F	9.8	12.171	.1140
	-G	9.7	11.656	.0696
	-H	9.8	12.385	.1330
	-I	10.3	14.992	.1391
	-J	10.6	17.158	.2454
	x	10.6	16.498	.1688
	s	.7	3.705	.0669
3	-A	10.5	18.829	.2035
	-B	9.2	19.091	.1520
	-C	10.0	16.576	.1684
	-D	11.0	14.285	.3268
	-E	11.5	18.001	.1516
	-F	10.5	19.530	.2068
	-G	11.8	22.749	.2713
	-H	10.7	20.394	.2365
	-I	10.1	18.037	.2667
	-J	11.4	16.016	.4872
	x	10.7	18.351	.2471
	s	.7	2.259	.0964

Continued on next page.

Table A1.2 Continued Day 42 Pallant Creek Hatchery Fish

SAMPLE		FORK (cm)	FISH WEIGHT (g)	LIVER (g)
4	-A	11.1	18.782	.1965
	-B	11.2	20.362	.1594
	-C	10.2	15.233	.1855
	-D	10.7	11.978	.1293
	-E	10.6	18.277	.2098
	-F	10.4	14.545	.1540
	-G	11.2	21.138	.2861
	-H	11.7	22.027	.2252
	-I	11.6	21.405	.2676
	-J	9.9	13.064	.1551
	x	10.9	17.681	.1969
	s	.6	3.509	.0486
5	-A	10.8	16.562	.1719
	-B	10.8	15.930	.1691
	-C	10.1	13.520	.1153
	-D	10.1	14.823	.2071
	-E	10.3	18.668	.1695
	-F	11.6	21.488	.3128
	-G	10.2	15.261	.1172
	-H	10.4	18.869	.1405
	-I	9.7	12.819	.1860
	-J	10.3	20.724	.2387
	x	10.4	16.866	.1828
	s	.5	2.807	.0564
6	-A	10.3	17.900	.2326
	-B	9.2	14.344	.1140
	-C	10.5	20.938	.2327
	-D	10.2	15.814	.1642
	-E	10.7	18.837	.2665
	-F	10.3	17.487	.1914
	-G	11.0	23.960	.3938
	-H	10.5	18.373	.2198
	-I	8.9	10.641	.0889
	-J	9.5	13.266	.2089
	x	10.1	17.156	.2113
	s	.6	3.645	.0803

Continued on next page.

Table A1.2 Continued Day 42 Pallant Creek Hatchery Fish

SAMPLE		FORK (cm)	FISH WEIGHT (g)	LIVER (g)
7	-A	11.2	24.074	.3334
	-B	10.2	17.029	.1404
	-C	9.6	15.471	.2118
	-D	9.3	12.753	.1350
	-E	11.2	19.660	.2111
	-F	.4	1.321	.0428
	-G	10.0	18.021	.2651
	-H	10.5	16.478	.1799
	-I	9.5	18.253	.4291
	-J	10.3	15.312	.2046
	x	9.2	15.837	.2153
	s	3.0	5.617	.1026
8	-A	10.4	16.420	.2074
	-B	9.7	11.599	.0990
	-C	9.0	9.672	.1627
	-D	10.7	18.071	.2053
	-E	10.8	16.558	.1404
	-F	10.8	15.969	.1278
	-G	11.6	23.497	.2352
	-H	11.8	21.692	.2372
	-I	11.3	20.977	.1824
	-J	11.5	23.191	.2684
	x	10.8	17.765	.1866
	s	.8	4.451	.0512
9	-A	11.5	20.590	.3144
	-B	11.2	21.334	.1970
	-C	10.2	16.067	.1736
	-D	10.6	16.081	.1933
	-E	9.9	13.519	.1825
	-F	9.9	13.249	.1256
	-G	11.0	21.796	.3743
	-H	10.6	17.512	.2193
	-I	11.1	15.775	.1366
	-J	10.5	18.649	.2790
	x	10.7	17.457	.2196
	s	.5	2.915	.0754

Continued on next page.

Table A1.2 Continued Day 42 Pallant Creek Hatchery Fish

SAMPLE		FORK (cm)	FISH WEIGHT (g)	LIVER (g)
10	-A	10.9	17.657	.1910
	-B	10.5	13.899	.0738
	-C	10.5	16.447	.1413
	-D	10.0	13.635	.1320
	-E	10.5	14.526	.1062
	-F	10.5	16.706	.1323
	-G	9.5	10.878	.1240
	-H	10.0	13.095	.1932
	-I	9.8	13.216	.1312
	-J	11.6	22.989	.3601
	x	10.4	15.305	.1585
	s	.6	3.195	.0751
11	-A	10.8	14.622	.1601
	-B	10.6	19.982	.2363
	-C	10.8	15.341	.1243
	-D	10.9	21.199	.2259
	-E	10.8	19.573	.2576
	-F	10.1	14.804	.1428
	-G	10.0	14.195	.1577
	-H	11.3	23.414	.2171
	-I	11.2	21.795	.2141
	-J	10.6	17.688	.1994
	x	10.7	18.261	.1935
	s	.4	3.208	.0421
12	-A	11.4	20.848	.2459
	-B	12.1	22.617	.2657
	-C	11.8	22.391	.1570
	-D	11.0	17.513	.2709
	-E	10.2	12.973	.0673
	-F	9.8	13.262	.1570
	-G	11.9	25.034	.2598
	-H	10.8	17.814	.1595
	-I	10.5	15.940	.2130
	-J	12.8	30.811	.3155
	x	11.2	19.920	.2112
	s	.9	5.269	.0709

Continued on next page.

Table A1.2 Continued Day 42 Pallant Creek Hatchery Fish

SAMPLE		FORK (cm)	FISH WEIGHT (g)	LIVER (g)
13	-A	11.0	20.823	.2440
	-B	11.9	22.572	.2026
	-C	11.8	22.623	.2730
	-D	11.1	18.894	.1515
	-E	10.6	20.270	.3225
	-F	10.6	17.320	.2522
	-G	10.8	16.422	.1750
	-H	10.6	18.670	.2715
	-I	10.7	15.919	.1935
	-J	11.5	20.447	.1828
	x	11.1	19.396	.2269
	s	.5	2.246	.0512
14	-A	10.9	16.945	.1339
	-B	10.7	16.919	.1617
	-C	10.9	19.705	.2901
	-D	9.7	12.353	.1317
	-E	11.0	19.540	
	-F	12.0	22.801	.2058
	-G	10.7	16.543	.1704
	-H	11.2	21.013	.2673
	-I	10.9	18.620	.2638
	-J	10.3	15.583	.1000
	x	10.8	18.002	.1916
	s	.6	2.827	.0645
15	-A	10.5	15.515	.1112
	-B	10.5	15.151	.1260
	-C	11.2	22.273	.3072
	-D	10.7	16.599	.2470
	-E	11.5	19.747	.1843
	-F	10.9	18.540	.2426
	-G	11.1	19.771	.2246
	-H	11.3	18.260	.1594
	-I	10.4	15.579	.1350
	-J	10.6	17.103	.2003
	x	10.9	17.854	.1938
	s	.4	2.180	.0593

Table A1.3 Day 42 Emplacement Fish: Middle Barbie Creek

SAMPLE		FORK (cm)	FISH WEIGHT (g)	LIVER (g)
16	-A	10.4	15.867	.1365
	-B	9.2	10.605	.1384
	-C	9.2	9.587	.1155
	-D	9.8	12.292	.1285
	-E	10.1	13.028	.1325
	-F	10.4	14.855	.1361
	-G	9.7	12.916	.0987
	-H	9.6	11.341	.0970
	-I	9.5	11.827	.0652
	-J	9.2	9.628	.0964
	x	9.7	12.195	.1145
	s	.4	1.964	.0231
17	-A	9.6	14.594	.2266
	-B	9.6	13.357	.1571
	-C	8.9	10.408	.1050
	-D	8.7	10.704	.1280
	-E	9.8	14.151	.1558
	-F	9.0	12.037	.1179
	-G	8.2	8.315	.0895
	-H	8.6	11.296	.1328
	-I	9.1	12.723	.2052
	-J	8.3	9.919	.1207
	x	9.0	11.750	.1439
	s	.5	1.890	.0412
18	-A	9.9	15.491	.1916
	-B	9.2	10.059	.0925
	-C	9.0	11.213	.1441
	-D	10.1	17.234	.1905
	-E	9.0	12.117	.1369
	-F	9.1	10.695	.1064
	-G	9.9	15.763	.2075
	-H	9.1	11.873	.1293
	-I	9.1	13.008	.1535
	-J	8.2	10.205	.1235
	x	9.3	12.766	.1476
	s	.5	2.413	.0363

Continued on next page.

Table A1.3 Continued. Day 42 Emplacement Fish: Middle Barbie Creek

SAMPLE		FORK (cm)	FISH WEIGHT (g)	LIVER (g)
19	-A	10.4	14.013	.1406
	-B	9.3	11.102	.0909
	-C	10.1	12.869	.0976
	-D	9.6	11.157	.1255
	-E	9.2	10.075	.0693
	-F	9.7	12.892	.1183
	-G	9.8	12.473	.1016
	-H	10.0	14.309	.1311
	-I	10.8	15.015	.1151
	-J	10.2	14.900	.1860
	x	9.9	12.881	.1176
	s	.5	1.616	.0303
20	-A	9.6	11.526	(.1454)
	-B	9.4	9.723	.1269
	-C	9.8	12.337	.1079
	-D	10.1	13.150	.0993
	-E	10.0	12.726	.1110
	-F	10.7	15.966	.1069
	-G	9.4	10.145	.0744
	-H	9.6	10.717	.0523
	-I	10.0	12.966	.1020
	-J	10.4	14.270	.0487
	x	9.9	12.353	.0922
	s	.4	1.817	.0258

Table A1.4 Day 42 Emplacement Fish: Lower Barbie Creek

SAMPLE		FORK (cm)	FISH WEIGHT (g)	LIVER (g)
22	-A	9.5	9.608	.0755
	-B	8.5	6.564	.0696
	-C	10.0	13.071	.1300
	-D	9.8	11.372	.1220
	-E	9.3	9.813	.0880
	-F	9.6	12.758	.1271
	-G	9.6	13.180	.1711
	-H	9.7	12.355	.1370
	-I	9.8	10.175	.0871
	-J	9.4	10.293	.1169
	x	9.5	10.919	.1124
	s	.4	1.956	.0302
23	-A	9.4	11.761	.1327
	-B	8.7	11.254	.1221
	-C	9.4	12.513	.1451
	-D	9.0	9.572	.1281
	-E	8.4	11.866	.1308
	-F	9.1	11.915	.1682
	-G	9.2	10.931	.1179
	-H	9.4	14.205	.2174
	-I	9.6	13.481	.2287
	-J	9.7	13.606	.2249
	x	9.2	12.110	.1616
	s	.4	1.321	.0428
24	-A	10.1	12.402	.1076
	-B	9.9	13.031	.1506
	-C	9.8	11.236	.0843
	-D	9.2	8.677	.0798
	-E	10.0	12.464	.1339
	-F	9.5	10.745	.1138
	-G	10.3	12.330	.1293
	-H	10.3	14.387	.1436
	-I	10.2	11.904	.0772
	-J	9.0	8.546	.1076
	x	9.8	11.572	.1128
	s	.4	1.748	.0251

Continued on next page.

Table A1.4 Continued Day 42 Emplacement Fish: Lower Barbie Creek

SAMPLE		FORK (cm)	FISH WEIGHT (g)	LIVER (g)
25	-A	9.4	10.402	.1260
	-B	10.2	12.222	.1228
	-C	10.0	13.324	.1661
	-D	10.1	13.608	.2002
	-E	9.1	10.711	.1555
	-F	9.6	13.839	.2165
	-G	10.2	15.525	.2622
	-H	9.7	12.929	.1992
	-I	9.1	9.729	.1578
	-J	9.6	14.719	.1748
	x	9.7	12.701	.1781
	s	.4	1.814	.0404
26	-A	9.6	10.122	.0503
	-B	9.7	11.455	.0677
	-C	9.3	10.927	.0666
	-D	9.1	8.967	.0701
	-E	8.7	9.592	.0803
	-F	10.5	15.870	.1515
	-G	9.2	9.329	.0414
	-H	9.8	11.276	.0916
	-I	9.3	10.646	.1060
	-J	8.6	8.006	.0666
	x	9.4	10.619	.0792
	s	.5	2.033	.0298

Table A1.5 Day 42 Emplacement Fish: Gold Creek

SAMPLE		FORK (cm)	FISH WEIGHT (g)	LIVER (g)
28	-A	9.2	11.576	.1354
	-B	8.3	8.914	.1055
	-C	9.2	11.211	.1281
	-D	8.0	9.196	.1248
	-E	9.1	11.816	.1293
	-F	10.0	16.701	.1833
	-G	9.5	13.482	.1359
	-H	8.8	10.883	.0846
	-I	10.3	15.514	.1814
	-J	8.8	11.326	.1259
	x	9.1	12.062	.1334
	s	.7	2.378	.0285
29	-A	9.4	9.478	.0853
	-B	9.6	11.945	.0756
	-C	9.6	12.102	.1168
	-D	9.8	11.911	.1339
	-E	9.5	9.844	.0742
	-F	10.6	15.625	.1357
	-G	9.6	10.955	.0683
	-H	9.2	9.944	.0870
	-I	11.0	17.235	.1695
	-J	10.4	15.866	.0917
	x	9.9	12.491	.1038
	s	.6	2.635	.0318
30	-A	9.0	10.818	.0990
	-B	9.3	12.191	.1622
	-C	9.6	13.457	.1576
	-D	9.8	12.858	.1733
	-E	9.2	11.345	.1069
	-F	8.8	12.235	.1711
	-G	9.5	12.448	.1322
	-H	8.5	10.043	.1252
	-I	9.4	11.911	.1232
	-J	9.9	13.931	.1952
	x	9.3	12.124	.1446
	s	.4	1.113	.0301

Continued on next page.

Table A1.5 Continued Day 42 Emplacement Fish: Gold Creek

SAMPLE		FORK (cm)	FISH WEIGHT (g)	LIVER (g)
31	-A	10.1	13.853	.1433
	-B	10.3	14.834	.1220
	-C	10.7	14.727	.1214
	-D	10.7	15.055	.1446
	-E	9.8	12.356	.1317
	-F	10.9	15.971	.1017
	-G	9.7	11.593	.0812
	-H	10.1	10.802	.1097
	-I	9.2	9.358	.0633
	-J	9.2	13.100	.1415
	x	10.1	13.165	.1160
	s	.6	2.009	.0260
32	-A	9.2	11.357	.1046
	-B	9.7	13.774	.1658
	-C	9.4	12.388	.1455
	-D	9.2	11.044	.1086
	-E	9.0	11.202	.1373
	-F	9.5	11.445	.1239
	-G	10.7	17.355	.1864
	-H	9.9	15.136	.1694
	-I	9.9	12.702	.1659
	-J	10.0	14.306	.1703
	x	9.7	13.071	.1478
	s	.5	1.960	.0268

Table A1.6 Feral Fish: Middle Barbie Creek

SAMPLE		FORK (cm)	FISH WEIGHT (g)	LIVER (g)
45	-A	8.3	9.907	.1056
	-B	8.1	8.454	.0889
	-C	8.7	8.402	.1664
	-D	8.0	10.606	.0969
	-E	7.2	5.493	.0548
	-F	7.9	9.703	.1084
	-G	7.8	8.093	.0761
	-H	9.0	9.518	.1480
	-I	8.0	7.586	.0721
	-J	7.6	6.617	.0316
	x	8.1	8.438	.0949
	s	.5	1.499	.0384
46	-A	8.4	7.739	.0620
	-B	8.5	7.004	.0061
	-C	9.2	10.310	.0801
	-D	8.5	7.006	.0593
	-E	8.8	8.391	.0519
	-F	8.6	7.712	.0660
	-G	7.9	6.911	.0637
	-H	8.0	5.966	.0441
	-I	7.3	4.853	.0408
	-J	9.4	11.376	.0808
	x	8.5	7.727	.0555
	s	6	1.830	.0207

Table A1.7 Feral Fish: Lower Barbie Creek

SAMPLE		FORK (cm)	FISH WEIGHT (g)	LIVER (g)
34	-A	8.2	10.908	.1055
	-B	7.6	7.168	.0948
	-C	9.3	13.294	.0920
	-D	8.1	8.274	.0504
	-E	7.7	7.703	.0652
	-F	7.5	6.523	.0479
	-G	9.0	10.954	.1095
	-H	7.8	7.337	.0635
	-I	8.1	11.400	.7840
	-J	8.7	10.878	.1162
	x	8.2	9.444	.1529
	s	.6	2.184	.2117
35	-A	7.6	7.412	.0807
	-B	7.4	6.601	.0584
	-C	7.7	8.046	.0753
	-D	8.1	9.963	.0665
	-E	8.1	9.043	.0291
	-F	8.1	8.782	.0775
	-G	7.3	7.325	.0789
	-H	7.6	9.336	.0863
	-I	8.4	.11.471	.0799
	-J	7.5	8.537	.0984
	x	7.8	8.652	.0731
	s	.3	1.349	.0178
36	-A	9.0	9.948	.0749
	-B	9.0	9.770	.0955
	-C	8.6	8.807	.0585
	-D	8.1	8.163	.0656
	-E	8.5	10.026	.0920
	-F	8.4	7.855	.0450
	-G	8.2	10.660	.1562
	-H	8.6	7.917	.1036
	-I	8.1	7.440	.0685
	-J	8.1	6.829	.0678
	x	8.5	8.741	.0828
	s	.3	1.225	.0298

Continued on next page.

Table A1.7 Continued. Feral Fish: Lower Barbie Creek

SAMPLE		FORK (cm)	FISH WEIGHT (g)	LIVER (g)
37	-A	8.4	10.162	.0581
	-B	8.5	9.459	.0704
	-C	8.2	8.656	.0686
	-D	7.8	7.394	.0531
	-E	8.3	9.102	.0623
	-F	8.8	10.978	.1342
	-G	8.3	8.954	.0945
	-H	7.9	9.857	.1570
	-I	7.8	8.379	.0665
	-J	8.7	15.708	.2074
	x	8.3	9.865	.0972
	s	.3	2.164	.0493
38	-A	8.0	8.212	.0521
	-B	8.1	10.603	.0900
	-C	7.6	7.802	.0951
	-D	8.6	9.234	.0596
	-E	7.2	7.899	.0500
	-F	8.2	8.043	.1061
	-G	8.0	9.079	.0680
	-H	8.1	10.233	.0940
	-I	8.0	8.842	.0937
	-J	7.7	7.895	.0737
	x	8.0	8.784	.0782
	s	.4	.955	.0191
43	-A	8.2	6.953	.0751
	-B	8.2	6.544	.0461
	-C	8.4	7.976	.0952
	-D	7.9	6.021	.0680
	-E	8.2	6.966	.0743
	-F	8.1	7.484	.0698
	-G	9.1	9.235	.0967
	-H	8.3	7.653	.0612
	-I	9.0	8.308	.0521
	-J	8.0	6.504	.0636
	x	8.3	7.364	.0702
	s	.4	.917	.0155

Continued on next page.

Table A1.7 Continued. Feral Fish: Lower Barbie Creek

SAMPLE		FORK (cm)	FISH WEIGHT (g)	LIVER (g)
44	-A	7.5	6.474	.1025
	-B	9.0	10.824	.1411
	-C	7.4	8.341	.0750
	-D	7.5	7.715	.1166
	-E	8.3	10.443	.1387
	-F	8.2	10.009	.1000
	-G	8.3	9.556	.1266
	-H	8.4	7.436	.0744
	-I	7.2	7.738	.0813
	-J	8.7	11.000	.1690
	x	8.1	8.954	.1125
	s	.6	1.524	.0300

Table A1.8 Feral Fish: Gold Creek

SAMPLE		FORK (cm)	FISH WEIGHT (g)	LIVER (g)
40	-A	8.6	8.184	.0464
	-B	10.1	11.495	.0898
	-C	9.7	11.329	.0976
	-D	9.6	9.416	.1153
	-E	9.1	9.063	.0833
	-F	10.9	13.772	.1438
	-G	8.0	6.859	.0679
	-H	9.6	8.189	.0952
	-I	8.7	9.469	.0654
	-J	9.4	9.161	.0821
	x	9.4	9.694	.0887
	s	.8	1.896	.0259
41	-A	9.5	12.936	.1549
	-B	7.2	6.392	.0524
	-C	9.6	14.124	.1581
	-D	8.0	8.643	.0869
	-E	8.2	10.789	.1100
	-F	8.6	10.155	.0632
	-G	8.8	11.431	.1055
	-H	8.0	9.167	.1191
	-I	8.1	8.175	.0730
	-J	8.3	8.502	.0734
	x	8.4	10.031	.0997
	s	.7	2.224	.0348
48	-A	7.1	4.997	.0412
	-B	7.6	5.089	.0376
	-C	8.0	6.099	.0431
	-D	7.7	5.470	.0168
	-E	7.8	5.467	.0555
	-F	7.6	6.049	.0440
	-G	8.0	6.370	.0466
	-H	8.8	7.795	.0487
	-I	7.5	5.325	.0616
	-J	7.2	5.155	.0541
	x	7.6	5.577	.0454
	s	.5	.827.	.0113

APPENDIX 2

Normalization of Metallothionein Data

Table A2.1A Normalized Metallothionein Data: Day 0 Pallant Creek Hatchery Fish

SAMPLE GROUP	MT	A	B	C	D
		(all values ug/mg wet liver tissue)			
1	.373	1.016 (.023)[a]	.404	107.3	.425
2	.274	.952	.380	108.3	.395
3	.325			119	
4	.234	1.315 (.674)	.196	118	.188
5	.241	.784	.338	119.5	.319
6	.198	1.217 (.576)	.179	104.5	.193
7	.249	1.320 (.569)	.208	113.3	.207
x	.271	1.101	.284	112.7	.288
s	.055	.199	.092	65.6	.097

NOTES:
A) Thiolic protein.
B) MT normalized with respect to thiolic protein (A).
C) Average Total Protein.
D) MT normalized with respect to both A and C
a) Parenthesized values indicate standard deviation of triplicate analyses.

Table A2.1B Normalized Metallothionein Data: Day 42 Pallant Creek Hatchery Fish

SAMPLE GROUP	MT	A	B	C	D
	(all values ug/mg wet liver tissue)				
REFERENCE (NO TRANSPORT)					
1	.332	1.271	.336	98.0	.307
2	.309	1.251	.317	95.0	.300
3	.246	1.052	.300	79	.341
	(.017)[a]	(.037)			
4	.243	1.542	.203	90.8	.200
5	.177	1.308	.174	85.6	.182
x	.261	1.285	.266	89.7	.266
s	.055	.156	.065	6.8	.063
TRANSPORT (AMBIENT T)					
6	.366	1.268	.396	92.6	.454
7	.243	1.562	.214	81.4	.184
8	.388	1.424	.374	88.8	.472
9	.249	1.340	.255	82	.224
10	.263	1.271	.284	77.4	.278
x	.302	1.373	.305	84.4	.309
s	.062	.110	.070	5.5	.061

Continued on next page.

Table A2.1B Continued Normalized Metallothionein Data: Day 42 Pallant Creek Hatchery Fish

TRANSPORT (ELEVATED T)					
11	.307	1.388	.294	62.6	.341
12	.378	1.434	.350	78.2	.326
13	.295	1.212	.323	69.4	.338
14	.234	1.276 (.063)	.244	85.1	.208
15	.280	1.336	.279	68.0	.298
x	.299	1.329	.298	72.7	.302
s	.047	.079	.036	8.0	.049
OVERALL					
x	.287	.290	.313	82.3	.292
s	.058	.061	.120	9.9	.061

NOTES:
A) Thiolic protein.
B) MT normalized with respect to thiolic protein (A).
C) Average Total Protein.
D) MT normalized with respect to both A and C
a) Parenthesized values indicate standard deviation of triplicate analyses.

Table A2.1C Normalized Metallothionein Data: Emplacement Fish

SAMPLE GROUP	MT	A	B	C	D
		(all values ug/mg wet liver tissue)			
MIDDLE BARBIE CREEK					
16	.295	.978	.303	76.0	.299
17	.2576	.932	.284	68.9	.309
18	.274	1.244	.226	84.9	.199
19	.223	.973 (.063)[a]	.235	68.3	.258
20	.266	.998	.273	77.0	.266
x	.263	1.025	.264	75.0	.266
s	.023	.111	.029	6.1	.039
LOWER BARBIE CREEK					
22	.277	1.005	.314	80.3	.316
23	.323	1.186	.310	77.6	.376
24	.233	1.073	.247	65.0	.258
25	.282	1.232	.260	81.3	.263
26	.271	1.196	.258	81.0	.252
x	.277	1.138	.278	77.0	.269
s	.029	.085	.028	6.2	.029
GOLD CREEK					
28	.260	1.065	.259	70.1	.270
29	.170	1.024	.176	68.2	.189
30	.241	1.035	.247	76.5	.236
31	.151	1.078	.149	72.7	.149
32	.247	1.101	.238	77.8	.209
x	.214	1.061	.214	73.0	.211
s	.044	.028	.043	3.8	.041
OVERALL					
x	.251	1.075	.252	75.0	.249
s	.043	.095	.044	5.7	.045

NOTES:
A) Thiolic protein.
B) MT normalized with respect to thiolic protein (A).
C) Average Total Protein.
D) MT normalized with respect to both A and C
a) Parenthesized values indicate standard deviation of triplicate analyses.

Table A2.1D Normalized Metallothionein Data: Feral Fish

SAMPLE GROUP	MT	A	B	C	D
		(all values ug/mg wet liver tissue)			
LOWER BARBIE CREEK AUG 17/88					
43	.190	1.027	.185	80.5	.178
44	.177	.936	.177	87.1	.158
MIDDLE BARBIE CREEK AUG 17/88					
45	.150	.975	.154	76.0	.154
46	.155	.923	.168	76.8	.170
LOWER BARBIE CREEK SEPT 88					
34	.091	.604	.151	53.3	.219
35	.172	.999	.172	83.1	.161
36	.167	1.009	.165	70.1	.183
37	.201	.971	.207	69.8	.230
38	.191	1.030	.185	83.5	.206
GOLD CREEK SEPT 88					
40	.205	1.106	.185	73.7	.195
41	.160	1.119	.160	97.4	.127
AUG 17/88					
48	.257	1.287	.199	79.8	.194
OVERALL					
x	.176	.999	.176	77.6	.182
s	.038	.152	.017	10.4	.028

NOTES:
A) Thiolic protein.
B) MT normalized with respect to thiolic protein (A).
C) Average Total Protein.
D) MT normalized with respect to both A and C
a) Parenthesized values indicate standard deviation of triplicate analyses.

APPENDIX F - CBR INTERNATIONAL (ii) SUMMARY OF LIVER PROTEIN - CAGED FISH

APPENDIX F(ii) - SUMMARY OF LIVER PROTEIN-CAGED FISH

TOTAL PROTEIN (HOMOGENATE)(ug/g wet liver tissue)

STATION	PALLANT HATCHERY		LOWER BARBIE	MID BARBIE	GOLD
	DAY-0	DAY-42	DAY-42	DAY-42	DAY-42
	111.0	87.7	81.5	77.2	72.9
	106.2	85.0	79.3	71.0	69.2
	115.8	82.3	52.8	91.9	84.0
	115.7	87.4	83.7	69.9	66.5
	105.6	81.6	84.9	83.4	78.5
	98.5				
	99.3				
mean	107.4	84.8	76.4	78.7	74.2
sd	6.6	2.5	12.0	8.2	6.3
n	7	5	5	5	5
rsd	6	3	16	10	9

TOTAL PROTEIN (CYTOSOL)(ug/g wet liver tissue)

STATION	PALLANT HATCHERY		LOWER BARBIE	MID BARBIE	GOLD
	DAY-0	DAY-42	DAY-42	DAY-42	DAY-42
	103.5	108.3	79.3	74.8	67.2
	110.5	105.0	76.0	66.8	67.2
	121.4	75.7	77.2	77.8	68.9
	119.3	94.3	78.8	66.7	78.8
	133.4	89.6	77.1	70.6	77.1
	110.4				
	127.2				
mean	118.0	94.6	77.7	71.3	71.8
sd	9.7	11.6	1.2	4.4	5.1
n	7	5	5	5	5
rsd	8	12	2	6	7

APPENDIX F(ii) - SUMMARY OF LIVER PROTEIN-CAGED FISH

METALLOTHIONEIN (ug/g wet liver tissue)

STATION	PALLANT HATCHERY		LOWER BARBIE	MID BARBIE	GOLD
	DAY-0	DAY-42	DAY-42	DAY-42	DAY-42
	0.373	0.332	0.277	0.295	0.260
	0.274	0.309	0.323	0.257	0.170
	0.325	0.246	0.233	0.274	0.241
	0.234	0.243	0.282	0.223	0.151
	0.241	0.177	0.271	0.266	0.247
	0.198				
	0.249				
mean	0.271	0.261	0.277	0.263	0.214
sd	0.055	0.055	0.029	0.024	0.044
n	7	5	5	5	5
rsd	20	21	10	9	21

THIOLIC (ug/g wet liver tissue)

STATION	PALLANT HATCHERY		LOWER BARBIE	MID BARBIE	GOLD
	DAY-0	DAY-42	DAY-42	DAY-42	DAY-42
	1.016	1.271	1.005	0.978	1.065
	0.952	1.251	1.186	0.932	1.024
		1.052	1.073	1.244	1.035
	1.315	1.542	1.232	0.973	1.078
	0.784	1.308	1.196	0.998	1.101
	1.217				
	1.320				
mean	1.101	1.285	1.138	1.025	1.061
sd	0.199	0.156	0.085	0.112	0.028
n	6	5	5	5	5
rsd	18	12	7	11	3

APPENDIX F - CBR INTERNATIONAL (iii) SUMMARY OF LIVER PROTEIN - FERAL FISH

APPENDIX F(iii) - SUMMARY OF LIVER PROTEIN - FERAL FISH

TOTAL PROTEIN (HOMOGENATE)(ug/g wet liver tissue)

STATION	LOWER BARBIE	MID BARBIE	GOLD
AUG 17/88	76.2	71.7	77.1
	80.8	66.4	92.3
SEP 15/88	56.5		82.3
	90.4		
	70.2		
	77.6		
	83.1		
mean	76.4	69.0	83.9
sd	10.0	2.7	6.3
n	7	2	3
rsd	13	4	8

TOTAL PROTEIN (CYTOSOL)(ug/g wet liver tissue)

STATION	LOWER BARBIE	MID BARBIE	GOLD
AUG 17/88	84.4	80.4	70.3
	93.4	87.1	102.6
SEP 15/88	50.0		85.0
	75.8		
	70.0		
	62.0		
	83.8		
mean	74.2	83.8	86.0
sd	13.7	3.3	13.2
n	7	2	3
rsd	19	4	15

APPENDIX F(iii) - SUMMARY OF LIVER PROTEIN - FERAL FISH

METALLOTHIONEIN (ug/g wet liver tissue)

STATION	LOWER BARBIE	MID BARBIE	GOLD
AUG 17/88	0.190	0.150	0.205
	0.177	0.155	0.160
SEP 15/88	0.091		0.207
	0.172		
	0.167		
	0.201		
	0.191		
mean	0.170	0.152	0.191
sd	0.034	0.003	0.022
n	7	2	3
rsd	20	2	11

THIOLIC (ug/g wet liver tissue)

STATION	LOWER BARBIE	MID BARBIE	GOLD
AUG 17/88	1.027	0.975	1.106
	0.936	0.923	1.119
SEP 15/88	0.604		1.171
	0.999		
	1.009		
	0.971		
	1.030		
mean	0.939	0.949	1.132
sd	0.140	0.026	0.028
n	7	2	3
rsd	15	3	2

APPENDIX G - HETEROTROPHIC BACTERIA PLATE COUNT

APPENDIX G - HETEROTROPHIC BACTERIA PLATE COUNT

Surface or Spread Plate Method

Generally, the technique requires an aliquot of sample or a serial dilution of the sample being placed on a dried agar surface followed by absorption of the liquid and an incubation period.

Dilutions (using buffered distilled water) of the sample are set to ensure that some petri plates will contain between 30 and 300 colonies. An aliquot of 1.0ml of each dilution is spread on the plates. Analyses are conducted in duplicate and the reportable results are averaged and expressed in Colony Forming Units (CFU) per ml or gm.

Media (Heterotroph Plate Count Agar)

Peptone	3.0g
K_2HPO_4	0.2g
$MgSO_4$	0.05g
$FeCl_3$	0.001g
Soluble Casein	0.5g
Agar	15.0g
Distilled water	1.0L

Soak and dissolve ingredients in distilled water for 15 minutes, then bring to a boil to dissolve. Adjust pH to 7.2 after autoclaving (104kPa/20 minutes). Cool to 42-46°C. and pour into sterile petri dishes. Plates may be kept up to 4 weeks at 2-6°C. in a humidified refrigerator.